VOLUME 19 NUMBER 4

NUTRITION REVIEWS

April 1961

Activities of the Food and Nutrition Board, 1960, by LeRoy Voris... 97

Clinical Nutrition

Spontaneous Variations in Serum Lipids and Cholesterol..... 100
Levels of Vitamins A and E in Human Blood..... 101
Growth Hormone Studies in Man..... 103
Bitot's Spots..... 104

Experimental Nutrition

Hereditary and Infectious Components of Tooth Decay..... 105
Atherosclerosis in Miniature Pigs..... 108
Sex Differences in Susceptibility to Vitamin K Deficiency..... 110
Zinc Deficiency in Chicks..... 111
Dietary Protein and Acetate-1-C^{14} Incorporation..... 113
Fatty Acid Uptake by Skeletal Muscle..... 115
Antibody Formation by Vitamin Deficient Rats..... 116
Linoleic Ester and Cholesterol..... 117
Kwashiorkor and Plant Proteins..... 118
Pyridoxine and Transaminases..... 120
Vitamin B_6 and B_{12} Relationships..... 122
Injury, Growth, and Nitrogen Balance..... 123
Fluoride Content of Skeletal Tissues..... 124

Notes

Liver Enzymes in Glycogen Storage Disease..... 127
Fatty Acid Chain Length and Essential Fatty Acid Deficiency.. 127
Recent Books..... 128

Published Monthly at Mt. Royal and Guilford Aves., Baltimore, Md.

for

THE NUTRITION FOUNDATION, INC.

99 PARK AVE. NEW YORK 16, N. Y.

COPYRIGHT, 1961 NUTRITION FOUNDATION INC., NEW YORK, U. S. A.

Editor

FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, One Shattuck Street, Boston, Mass

Assistant Editor

PRISCILLA GOREE, One Shattuck Street, Boston.

Associate Editors

GEORGE K. DAVIS, PH.D., Department of Animal Husbandry and Nutrition, University of Florida, Gainesville.
DR. GILBERT B. FORBES, Department of Pediatrics, University of Rochester School of Medicine, Rochester.
ALFRED E. HARPER, PH.D., Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.
ROBERT E. HODGES, M.D., Department of Internal Medicine, State University of Iowa, Iowa City.
STANLEY LEVEY, PH.D., Departments of Biochemistry and Surgery, College of Medicine, Western Reserve University, Cleveland.
JAMES F. MEAD, PH.D., Department of Physiological Chemistry, School of Medicine, University of California, Los Angeles.
OLAF MICKELSEN, PH.D., Laboratory of Biochemistry and Nutrition, National Institute of Arthritis and Metabolic Diseases, Bethesda.
WILLIAM J. PYLES, M.D., St. Luke's Hospital, New York.
JAMES H. SHAW, PH.D., Schools of Dental Medicine and Public Health, Harvard University, Boston.
ELMER H. STOTZ, PH.D., Department of Biochemistry, School of Medicine, University of Rochester, Rochester.
DONALD M. WATKIN, M.D., Organización Panamericana de la Salud, Mexico City.
ROBERT W. WISSLER, PH.D., M.D., Department of Pathology, University of Chicago.

Editorial Committee

WALLACE R. AYKROYD, M.D., University of London
CHARLES S. DAVIDSON, M.D., Boston City Hospital
CONRAD A. ELVEHJEM, PH.D., University of Wisconsin
IAGO GALDSTON, M.D., New York Academy of Medicine
WENDELL H. GRIFFITH, PH.D., University of California
CHARLES GLEN KING, PH.D., Nutrition Foundation
BERWYN F. MATTISON, M.D., American Public Health Assn.
L. A. MAYNARD, PH.D., Cornell University
HAZEL STIEBELING, PH.D., Institute of Home Economics, U.S.D.A.
LEROY VORIS, PH.D., National Research Council
WARREN WEAVER, PH.D., Alfred P. Sloan Foundation
JOHN B. YOUMANS, M.D., American Medical Association

Subscriptions should be sent to The Nutrition Foundation, Inc., 99 Park Ave., New York 16, N. Y.
Rate $4.00 per year in the United States and Canada; $4.50 in other countries
Changes of address should be sent to The Nutrition Foundation.
Second-class postage paid at Baltimore, Md.

Note: Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved.

NUTRITION REVIEWS

VOL. 19 APRIL 1961 No. 4

ACTIVITIES OF THE FOOD AND NUTRITION BOARD, 1960

One of the assigned duties of the National Academy of Sciences-National Research Council (NAS-NRC) is "to survey the broad possibilities of science" with regard to the public welfare. The NAS-NRC has performed this duty in the field of nutrition through a series of committees on animal nutrition in continuous operation since 1920 and, since 1940, through the Food and Nutrition Board for human nutrition.

The science of nutrition is itself a rapidly advancing field of knowledge and research but its "broad possibilities" for human welfare lead to an intricate interplay of domestic and international forces involving medical, agricultural, commercial, technological, socio-economic, legal, and cultural factors. The Food and Nutrition Board endeavors to resolve some of these forces under auspices which are independent of legal restrictions and prejudical pressures.

The following notes on activities during 1960 indicate the scope and nature of the work of the Food and Nutrition Board.

DOMESTIC PROBLEMS

In regard to domestic problems, there were several with respect to milk. In general the Board has not shown favoritism to any particular foods, but has indicated that its recommended dietary allowances could be obtained from many combinations and variations of food patterns. However, in this country, milk is a major item in the food supply, and the dairy industry is a dominant factor in the agricultural economy. Primarily through the efforts of nutritionists, people have developed a high degree of confidence in milk as a basic food with good nutritional attributes. In view of the progressive dependence on milk for a large portion of the daily nutrients, any unwarranted depreciation of confidence in milk as a food which affected its production and consumption was not considered desirable. The Board faced two problems which were affecting the public confidence in milk.

The first was the occurrence of radionuclides in foods resulting from fallout in nuclear weapons testing. Milk had been singled out for particular attention and, in some areas, it was publicly represented as being a hazard beyond justifiable limits. The Board reviewed this matter through its Committee on Milk and instigated further study by the Food Protection Committee. The Board agreed that the matter of radionuclides in foods is a currently active problem, but should be studied with regard to the *total* food supply.

The second problem faced was that of the policy of the Food and Drug Administration in regard to food additive tolerances in milk. The Board questioned the incompatibility of the regulations which established zero tolerances in milk, but did establish safe tolerance levels for residues in practically all other agriculturally produced foods. Certain residues are inevitable, and the zero tolerance regulations are beyond control. The Board requested the Food Protection Committee to develop the evidence for review by the Board in working toward correction of the present situation. A statement of the Board's opinion was transmitted to the Commissioner of the Food and Drug Administration in September with favorable effect.

In addition to the problems cited above, the Board, through its Committee on Milk, responded to two requests from the Office of the Quartermaster General: (1) on the comparative nutritive qualities of recombined milk and fresh pasteurized milk and (2) on the suitability and safety of recombined milk for use by the armed forces.

Three other domestic problems were given attention:

(1) The question of ways of improving the effectiveness of nutrition education was again raised for consideration. A move was made to instigate a study of methodology in nutrition education, that is, a research approach for analysis of factors that determine food habits and prejudices, and factors which may be useful in effecting changes.

(2) The problem of nutrition planning for special environmental conditions was extended to include requirements for space flight as well as the older problem of nutrition requirements for atomic attack shelters. This was referred to the Committee on Dietary Allowances for the attention of a special subcommittee.

(3) The need for revision of policy in regard to the addition of specific nutrients to foods was considered. The current policy on enrichment and fortification of foods which was jointly adopted by the American Medical Association's Council on Foods and Nutrition in 1953 has been criticized as being too restrictive. An *ad hoc* committee was appointed to review the policy and make recommendations to the Board.

COMMITTEE ACTIVITIES

The Committee on Dietary Allowances, reorganized under the Chairmanship of Dr. Wendell H. Griffith, has been aided by reports by other committes of the Board which have been published recently. These include *The Role of Dietary Fat in Human Health, NAS-NRC Publication No. 575*, and *Evaluation of Protein Nutrition, NAS-NRC Publication No. 711*. The Subcommittee on Calcium participated in a workshop on bone densitometry sponsored by the Nutrition Study Section of the National Institutes of Health in December 1959. The present opinion indicates that bone densitometry by present methodology is not useful in assessing calcium utilization. Efforts to get information on calcium content of human bones may be aided by the investigations on strontium-90. The subcommittee is continuing to gather knowledge relative to human calcium requirements.

The Committee on Infant Nutrition reported progress in (1) better definitions of criteria for evaluation of infant development, (2) review of nutrient requirements, and (3) pointing out needed research to solve present problems.

The Committee on Amino Acids, following its published report, *Evaluation of Protein Nutrition*, proposed, with the Board's endorsement, (1) to critically examine methods and terminology extant for expressing amino acid requirements, (2) to study amino acid proportionality patterns in tissues as affected by diet, (3) to evaluate amino acid requirements further, and (4) to explore methods for evaluation of optimal protein levels and the effects of excesses.

In November 1960 the Food Protection Committee received from the Food Law Institute, Inc. an award for "distinguished food law services to the American people." The efforts and influence of this committee in the establishment of rationality in consideration of problems of food additives have been outstanding. Two publications during 1960 were *Problems in the Evaluation of Carcinogenic Hazard from Use of Food Additives, NAS-NRC Publication No. 749* and *Principles and Procedures for Evaluating the Safety of Food Additives, NAS-NRC Publication No. 750*.

INTERNATIONAL ACTIVITIES

(1) The Committee on Protein Malnutrition's Program in support of research on development of protein resources in protein-deficient countries took on added significance when dry skim milk ceased to be surplus in October 1959 and distribution outside the USA was temporarily discontinued. This research is supported by a grant of $550,000 from the Rockefeller Foundation and closely integrated with the action programs of the United Nations Children's Fund (UNICEF) and the Food and Agriculture Organization

and the World Health Organization of the United Nations. Some 23 grants in 18 countries are currently active. Stimulated by the results of the committee's program, UNICEF appropriated an additional $300,000 to supplement the basic research by field trials, pilot plants, and acceptability tests.

In cooperation with the Nutrition Study Section of the National Institutes of Health, the investigators supported by the Committee on Protein Malnutrition met together with U.S. personnel in August to review the results and expedite successful productivity in 1961. The Conference on Protein Needs of Infants and Preschool Children involved 75 people, 33 being from 18 foreign countries. The Proceedings of the Conference, being prepared for publication, will summarize the progress around the world in development of satisfactory protein products derived from many sources, some of which were previously considered unsuitable for human use.

(2) The Committee on International Nutrition Programs was set up under the Chairmanship of Dr. L. A. Maynard with the primary objective of seeing that proper attention be given to nutrition in the multiple programs designed for technological aid and economic development under governmental, United Nations, or private organizations. Two meetings proved helpful in providing an exchange in viewpoints among representatives of the International Cooperation Administration, the National Institutes of Health, the Foreign Agricultural Service and the Foreign Research and Technical Programs Division of the U.S. Department of Agriculture, the Office of Science Adviser in the Department of State, the Office of the Surgeon General, the Office of International Relations of the NAS-NRC, the Interdepartmental Committee on Nutrition for National Defense, and others.

As a follow-up, the committee proposed a brochure which would point out the futility of programs that ignore the multiphasic nature of improving standards of living in underdeveloped countries and emphasize the necessity of cooperative relationships among programs of all specialities. In this setting, nutrition has an important position which must fit into health, economics, and agricultural programs in practice as well as preachment.

(3) The Food and Nutrition Board was requested to assume administrative responsibility for travel grants and to assist in the planning stages of the FAO International Conference on Marine Food Resources and World Nutrition to be held in Washington in September 1961. A U.S. Scientific Advisory Committee includes representatives of the Board, the NIH's Nutrition Study Section, UNICEF, and the Department of the Interior.

With these examples of the activities of the Food and Nutrition Board in evidence, it might be well to note another group concerned with international nutrition within the Academy-Research Council. This is the U.S. National Committee of the International Union of Nutritional Sciences. Members of this committee are nominated by the American Institute of Nutrition and are appointed by the President of the National Academy of Sciences. Ex-officio members are the President of the American Institute of Nutrition and representatives of pertinent groups in the NAS-NRC including the Food and Nutrition Board.

The Academy, through its Office of International Relations, has about 20 national committees which adhere to the international scientific bodies. Close liaison is maintained with the Office of the Science Adviser in the U.S. Department of State. Many matters of importance to both science and international affairs are transacted through the non-government channels of the NAS-NRC. In this respect, the U.S.A. has a large degree of freedom for international cooperation in surveying "the broad possibilities of science."

LeRoy Voris
Executive Secretary
Food and Nutrition Board

SPONTANEOUS VARIATIONS IN SERUM LIPIDS AND CHOLESTEROL

Hypercholesterolemic patients may have wide fluctuations in their serum cholesterol concentrations which may occur spontaneously or in response to specific stress.

Each month new reports appear concerning some aspect of lipid and cholesterol metabolism. Most of them imply some relationship between hypercholesterolemia and clinical cardiovascular disease. Many are designed to show potential merit of some form of treatment.

But a word of caution is indicated. Before complete reliance can be placed upon determinations of lipids, one must know how reproducible the chemical methods are and how much variation occurs in the patients over a period of time. Statistics which represent average values for one group of patients as compared with another do serve a useful purpose, but not with reference to an individual patient or to a small group of patients.

In a recent report S. Segall and A. H. Neufeld (*Canad. Med. Assn. J.* **83,** *521 (1960)*) presented some remarkable evidence to indicate that lipid metabolism may vary widely, particularly in patients with hypercholesterolemia. The authors selected data from ten patients whom they had observed for considerable lengths of time. These were men between the ages of 27 and 59 years who had both hypercholesterolemia and a history of clinical coronary artery disease. At monthly intervals or more often the patients were observed while pursuing their usual activities. In some cases the observations included an interval of hospitalization. Blood was drawn for determinations of cholesterol, phospholipids, Svedberg flotation, and alpha and beta lipoproteins. A control group of nine normal subjects was included for comparison.

Spontaneous fluctuations were remarkably great, especially with regard to cholesterol values. These changes occurred without respect to treatment, change of diet, unusual stress or intercurrent illness. Comparisons of values were made in three categories: two weeks or less, four weeks or less, and one year or less. The cholesterol values (determined by two standard techniques in duplicate) had a mean fluctuation of 27.7 per cent, 32.2 per cent, and 44.4 per cent for each of the three respective periods. Variations in the other lipids were also considerable. For example, a mean variation of 38.2 per cent occurred in the cholesterol/phospholipid ratio in a period of one year or less.

Two patients who were observed for several years had extreme fluctuations with lower values observed during periods of hospitalization. This occurred despite undoubted anxiety over the illness which had necessitated hospitalization.

The normal subjects were found to have variations in their lipids of much lesser magnitude than the hypercholesterolemic patients. This would indicate that the clinical methods were accurate and that the fluctuations were valid.

The authors suggested that investigators who base conclusions on a few determinations of cholesterol or other lipids done at relatively short intervals may be deceiving themselves. Certainly this word of caution would seem appropriate.

Similar observations have been made by another group (J. E. Peterson, A. A. Wilcox, M. I. Haley and R. A. Keith, *Circulation* **22,** *247 (1960)*). Having noted a spontaneous fluctuation of more than 200 mg. per 100 ml. in the serum cholesterol concentration of a few individuals, they designed a study as follows. Five healthy medical students were selected who had marked lability of serum cholesterol concentration, and five others were selected who had relative stability. The "labile" group was hospitalized and subjected to several test conditions while subsisting on a diet of milk, eggs, and vegetables providing 75 g. of protein, 100 g. of fat, and 300 g. of carbohydrate. Venous blood was collected by indwelling catheter

or venipuncture at hourly intervals from 6 A.M. to 10 P.M. and again at 2 A.M. The "stable" group of subjects was studied at a later date under similar circumstances.

During the first day no unusual procedures were performed. On the second day the subjects were exposed to cold (4°C) for 45 minutes while wearing minimal clothing. The third day was devoted to psychological testing which consisted of attaching elaborate equipment to the subject to record respiration, electrocardiograms, finger plethysmograms, and galvanic skin responses. In addition, a microphone and padded ear phones were attached to the subject in order to induce the frustration of delayed auditory feed-back set to provide a delay of 0.30 seconds in verbal responses.

The subjects were then asked to interpret 15 proverbs and to answer five simple medical questions in the presence of their instructors. The reaction of the subjects varied, some showing retardation of thought, slurring of speech, and unfinished words, while others reacted with excessive perspiration, peculiar mannerisms, blocked speech, and inability to interpret proverbs or to recall common information. On the fourth day no tests were done, but on the fifth day 0.6 mg. of epinephrine was given subcutaneously to each subject.

The results of these studies showed a surprising lability in the concentration of cholesterol in the serum of the "labile" group regardless of the form of stress applied. By contrast the fluctuations of the "stable" group were much less marked. It is not surprising that the "control" values for cholesterol were much higher in the "labile" group than in the "stable" group.

The results of these clinical studies demonstrate that some patients, particularly those with hypercholesterolemia, have a propensity to wide fluctuations in their serum cholesterol and other lipids without apparent change in external environment and in response to specific forms of stress.

LEVELS OF VITAMINS A AND E IN HUMAN BLOOD

Serum levels of vitamin A in men are higher than those in women, and for both sexes the levels increase with age. There is no significant difference between the sexes in serum carotene or vitamin E levels.

Although a considerable number of surveys have been carried out on the vitamin A levels in human beings, very few such surveys have included vitamin E. Z. A. Leitner, T. Moore, and I. M. Sharman (*Brit. J. Nutrition* **14,** *157 (1960)*) determined the levels of both of these vitamins in blood secured from 742 men and 526 women living in London. An unstated number of the male subjects were young men in good health who were examined for life insurance or for their fitness for hard work. Most of the male and female subjects, however, reported to the physician because of some complaint. Individuals who had symptoms suggestive of diseases of the heart, lungs, intestinal tract, liver or kidneys were excluded from the latter group. The subjects were mostly middle class, but a few were manual workers. Blood samples were collected over the period 1948 to 1957.

As had been observed earlier by M. S. Kimble (*J. Lab. Clin. Med.* **24,** *1055 (1939)*), men had higher serum vitamin A levels (174 I. U. per 100 ml.) than women (142 I. U.). There was no significant difference in the carotenoid levels (121 μg per 100 ml.) for men and for women (131 μg). The sex difference in the level of vitamin A was attributed to more efficient conversion of carotene to vitamin A by men (Leitner *et al., loc. cit.*).

The level of serum vitamin A in the males was found to increase from a value of 150

I. U. per 100 ml. for the 19-year old subjects and to 191 in the 50 to 59-year age group. There was a steady decline in the older age groups to 178 I. U. for the 70 to 79-year old subjects. In the women, the serum vitamin A level increased steadily from a value of 116 I. U. in the ten to 19-year old group and to 185 I. U. in the 70 to 79-year old group.

The average carotenoid levels were consistently slightly lower for the men at all ages than for the women. For the men there was a progressive increase from 96 μg per 100 ml. of serum in the 10 to 19-year old group to 126 μg in the 50 to 59-year old group. From the latter group to the 79-year old group there was a decrease to 107 μg. The average carotenoid level in the ten to 19-year old women was 140 μg per 100 ml. This value dropped to 120 in the 30 to 39-year group and then paralleled about 10 μg above the curve for the men in subsequent age groups.

The blood levels of vitamin A in the males showed no change from January through June. There was a progressive increase from the late summer through December. The women, on the other hand, showed no seasonal change in the vitamin A levels. Both sexes showed an increase in blood carotenoid levels for the period April through September, with lower values for October to March. To explain the above seasonal changes, the investigators proposed that during the summer the carotene secured from the abundant supply of vegetables is converted to vitamin A, which is first stored and then subsequently mobilized into the blood stream.

When the values for serum vitamin A were averaged for two-year periods from 1948 to 1957, there was a progressive rise for both men and women. For the men, this increase was from 155 I. U. per 100 ml. in 1948–9 to 200 in 1956–7. For each of the two-year periods, the number of subjects ranged from 125 to 176. Over the same period, the serum levels for the women increased from 130 to 150 I. U. No such increases were noted in the blood carotenoid levels.

The increase in blood levels of vitamin A for 1948 through 1957 was related to an increase in the supply of this vitamin in the British diet. Leitner *et al.* (*loc. cit*) quote a number of surveys published in the United States during the period 1941–1946 which indicate that the serum vitamin A levels in the latter country were approximately twice those observed among the English. By 1957 the gap between the British and the American serum vitamin A levels had been closed. This, however, was not true for the carotenoid levels since the American values were still almost twice those reported by the British.

The vitamin E level in the serum of the above subjects was 1.05 mg. per 100 ml. for men and 1.06 mg. for the women (Leitner, *Brit. J. Nutrition* **14,** *281 (1960)*). Approximately 70 per cent of the subjects had levels between 0.8 and 1.2 mg.

There was no discernible difference in blood levels for men and women at any age group. There was, however, an increase from 0.9 mg. per 100 ml. of serum in the subjects under 30 years of age to 1.1 in those over 69.

There was no seasonal variation in the serum levels. This was also true for the biennial periods from 1952 to 1957. (Vitamin E analyses were not done on the subjects examined during the first four years of the study.)

The studies of Leitner *et al.* provide average serum levels for carotene and vitamins A and E. Studies such as these raise a question as to the significance of the observed blood levels. Undoubtedly, the change in the serum vitamin A levels can be considered evidence for an improvement in the diets of the English since the termination of World War II. The relatively low levels observed two years after the end of the war cannot be used as an argument either for or against the existence of a generalized

vitamin deficiency in Great Britain at that time. Should other surveys of this nature be carried out, it is hoped that an additional effort will be made to secure objective criteria that permit an evaluation of the significance of the serum vitamin levels.

These studies show that the blood levels for vitamin A are higher in males than they are in females and that for both sexes they tend to increase with age. The outstanding observation was the increase in the blood vitamin A levels over the period 1948 to 1957. Toward the end of this period the difference in the blood vitamin A levels between individuals in the United States and those in Great Britain ceased to exist.

GROWTH HORMONE STUDIES IN MAN

Balance studies for sodium, potassium, calcium, and phosphorus were carried out in human subjects receiving human, pig or monkey pituitary growth hormones.

In contrast to other hormones, the pituitary growth hormone has no specific target organ and its site of action is still unknown. Other hormones react on specific organs such as the thyroid or the adrenal and their effects are rapidly demonstrable. The growth hormone, however, produces its diffuse effect on the entire body over a long period of time. In spite of this there have been reports (*Nutrition Reviews* **16,** *253 (1958)*) that growth hormones administered to human subjects have produced changes in nitrogen balance over relatively short periods of time.

Recently, J. C. Beck *et al.* (*Metabolism* **9,** *699 (1960)*) studied three different types of pituitary growth hormones (human, simian, and porcine) in man. Seven individuals received one or more types of growth hormones. Metabolic balance studies were carried out in regard to nitrogen, potassium, sodium, calcium, and phosphorus—both when patients were receiving the hormone and during control periods preceding and following the hormone. Three of the individuals studied had dwarfism associated either with a craniopharygioma or an earlier trauma. One individual had a hypophysectomy, one was listed as a normal individual (24 years old), one had osteoporosis, and one had chronic renal failure.

In different series the growth hormone was given for periods of six to 15 days depending upon the individual study. For each series a control period preceded the growth hormone study and a post-control period followed. Five of the seven individuals showed increased nitrogen retention when the human growth hormone was given, characterized by a decrease in the urinary and fecal nitrogen excretions. The patient with osteoporosis and the individual with chronic renal failure did not have increased nitrogen retention. Also, the nitrogen balances of the individuals receiving the growth hormone derived from the pig were much less positive than the balances in patients receiving hormone derived from either the monkey or the human pituitary. The decreased urinary nitrogen normally occurred by the second day after receiving the growth hormone and usually persisted for two days after the treatment ended.

Only three subjects deficient in pituitary hormone showed potassium retention when the human growth hormone was given. However, in the period immediately following the administration of the hormone most individuals had increased losses of potassium. The normal individual and the two patients who had no pituitary deficiencies did not show increased potassium retentions. Growth hormone derived from the monkey caused potassium retention in the individual studied.

After receiving the human growth hormone five of the subjects had indications

that they were retaining phosphorus. One individual showed positive balances on both the human growth hormone and that derived from the monkey. Early in the control period, following the administration of the growth hormone, most patients had some increase in phosphorus loss.

In spite of producing large positive nitrogen balances only one of the individuals, a patient with pituitary deficiency, had a positive calcium balance following the administration of the growth hormone. The other individuals showed no change or some decrease in calcium balance. However, most subjects showed a positive balance in the control period which followed the administration of the growth hormone.

In regard to sodium balances five of the subjects had, to some degree, positive sodium balances while receiving the growth hormone (3 to 60 mEq per day). However, in the control period which followed the administration of the hormone they all showed smaller degrees of sodium retention. In spite of the increased sodium retention there was no constant increase in the aldosterone excretion in the urine. Five of the subjects showed an elevated urine aldosterone level as compared to the control days, but there was no strong correlation between the sodium balance and aldosterone excretion. It was noted that the human growth hormone had a consistent diabetogenic effect in both dwarfs and the hypophysectomized patients.

One individual received human growth hormone from April 1958 through March 1959. During the year she grew one and three quarter inches, compared to a half inch in the previous 14 months.

These studies offer definite data, though on a limited group of individuals, concerning the effect of growth hormone on the metabolic changes in the calcium, phosphorus, sodium, and potassium balances. Such data are needed before one can draw realistic conclusions as to the effect of the hormone in man, particularly on nutrients other than protein.

BITOT'S SPOTS

Bitot's spots, once considered indicative of vitamin A deficiency, have been found to have no apparent relation to the concentration of vitamin A in the blood or to the occurrence of night blindness.

For many years nutritionists have regarded white foamy spots on the bulbar conjunctiva of patients as being indicative of clinical vitamin A deficiency. P. Bitot described these lesions in persons who had nutritional night blindness (*Gaz. Hebd. med. and chir.* **10,** *284 (1863)*). Since then many nutritional surveys have been conducted in persons who had xerophthalmia or other nutritional disorders of the eyes.

The relationship between lack of vitamin A in the diet and epidermal thickening has been well established. However, application of this principle to the conjunctivae seems to have been less correct. Pingueculae, pterygia and other forms of conjunctival thickening seemed to be related more closely to environmental exposure than to dietary deficiency. E. C. Robertson and A. L. Morgan (*J. Nutrition* **31,** *471 (1946)*) studied the effects of vitamin A or of a placebo on the thickness of human conjunctiva and could find no difference. B. M. Nicol (*Brit. J. Nutrition* **3,** *25 (1949)*) studied natives of Nigeria who were deficient in vitamin A. He found ocular disease, but did not observe Bitot's spots. J. E. Kirk and M. Chieffi (*Am. J. Clin. Nutrition* **1,** *37 (1952)*) gave large doses of vitamin A to a group of elderly patients who had conjunctival thickening.

They found no immediate effect, but after four months demonstrable improvement occurred. H. Pollack (*Metabolism* **5,** *231 (1956)*) conducted a comprehensive nutritional survey of inhabitants of the Far East. Although both vitamin A deficiency and Bitot's spots were encountered, the latter did not disappear following therapy with vitamin A. N. B. Yourish (*Am. J. Ophth.* **36,** *109 (1953)*) studied two army men who had Bitot's spots in spite of a good diet and apparent good health. Administration of 30,000 units of vitamin A daily for four to six weeks failed to effect any change in these lesions.

In spite of all this evidence to the contrary, many persons continue to regard Bitot's spots as being pathognomonic of vitamin A deficiency. R. A. Hetler (*J. Nutrition* **8,** *75 (1934)*) described the development of Bitot's spots in ten rabbits and one monkey after prolonged feeding of a diet deficient primarily in vitamin A. This undoubtedly gave great impetus to the concept that vitamin A deficiency was responsible for these lesions. W. J. Darby (*Pub. Health Reports* **75,** *738 (1960)*) cautioned against considering Bitot's spots as significant in nutritional surveys.

Further evidence against a cause-and-effect relationship between vitamin A and Bitot's spots was furnished by a recent report by D. Paton and D. S. McLaren (*Am. J. Ophth.* **50,** *568 (1960)*). The authors examined the eyes of 1790 Ethiopian school children and found Bitot's spots in 51 of them (3.3 per cent of the boys and 1.8 per cent of the girls). Night vision was tested in 244 of these children of whom 28 had Bitot's spots. No significant differences were found between the two groups. The concentrations of vitamin A and of carotene were measured in the sera of 76 students, 31 of whom had Bitot's spots. Again there was no correlation between the amount of vitamin A or carotene in the blood and the occurrence of Bitot's spots. The authors were unable to observe these subjects subsequent to the administration of vitamin A.

Pathologically Bitot's spots occur as round or elliptical grey-white lesions located in the temporal portion of the bulbar conjunctiva. Usually they occur bilaterally and are somewhat more common in children, especially in boys. The lesion is composed of keratinized epithelium, meibomian secretions, and of corynebacterium xerose.

The authors concluded that Bitot's spots could not be considered diagnostic of vitamin A deficiency although they do tend to occur more frequently in persons who are poorly nourished. This report and the several reviews of the subject of Bitot's spots should serve to dissuade investigators from employing this lesion as an indication of vitamin A deficiency.

HEREDITARY AND INFECTIOUS COMPONENTS OF TOOTH DECAY

Recent studies indicate that experimental dental caries is an infectious disease, but also that specific levels of caries susceptibility and resistance are inherited.

The multifactorial nature of the etiology of dental caries has complicated the investigation of this disease that afflicts practically every citizen in industrialized countries at some time during a lifetime. These factors include: a bacterial agent, the accumulation of fermentable carbohydrates on and around caries-susceptible tooth surfaces, the quality and quantity of saliva, the inheritance of a specific proneness to or resistance to the development of carious lesions, and the modification of the inherited susceptibility by nutritional means during tooth development.

Since the oral cavities of man and experimental laboratory subjects become con-

taminated with a plethora of microorganisms soon after birth, evaluation of the type or types of bacteria responsible for tooth decay has been extremely difficult. In actuality, absolute proof that dental caries was of bacterial origin was not available until F. J. Orland *et al.* (*J. Dent. Res.* **33,** *147 (1954)*) reported that germ-free (gnotobiotic) rats did not develop carious lesions, while comparable rats maintained in the usual laboratory circumstances on the same diet developed characteristic lesions. Inoculations of enterococci into otherwise germ-free rats resulted in the development of typical carious lesions (Orland *et al., J. Am. Dent. Assn.* **50,** *259 (1955)*).

More recently, R. J. Fitzgerald *et al.* (*J. Dent. Res.* **39,** *923 (1960)*) have reported that germ-free rats maintained on a coarse particle, high sugar diet did not develop dental caries, while comparable rats inoculated with a single strain of an oral streptococcus isolated from the rat developed gross carious lesions. These lesions were histologically indistinguishable from those seen in rats maintained under the normal contaminated circumstances.

P. H. Keyes (*Arch. Oral Biol.* **1,** *304 (1960)*) has presented data to indicate that among experimental animals, dental caries is an infectious and transmissible disease. In the first experiment, two groups of weanling hamsters were fed a caries-producing diet. The hamsters in one group were kept in small groupings by themselves, while those in the other groups were housed with older caries-active animals. After 35 days, the hamsters in the second group had large grossly observable carious lesions, while those in the first group were almost free of lesions. In the second experiment, several litters were divided into halves at seven days of age and for the remaining 14 days of the suckling period the mouths of one half of the offspring were inoculated with a smear of fecal material from caries-active hamsters. This experiment was not definitive because of the possibility of cross-contamination of littermates, but a tendency toward an increase in caries was noted among the inoculated subjects, which suggested that maternal feces might be the source of the caries-producing flora during the suckling period.

A third experiment was conducted in which one group of hamsters was grouped by itself with no source of gross contamination, a second group was exposed to caries-active hamsters, and the drinking water of the third group was contaminated with feces from caries-active hamsters. The first group developed few lesions or remained caries-free, while the hamsters in the second and third groups developed a high incidence of rapidly progressing lesions.

The fourth and fifth experiments were conducted to determine the influence of antibiotic feeding to the females on the caries activity of their offspring. Either penicillin or erythromycin was added to the caries-producing diet at levels of 100 mg. per kg. The caries activity of the females was depressed and their offspring that received no antibiotic in the diet had negligible caries activities while they were maintained free from gross contamination by caries-active hamsters. However, the littermates that were housed with caries-active hamsters soon reestablished the cariogenic flora and developed substantial levels of caries activity.

The sixth experiment was conducted with Osborne-Mendel rats to determine if the caries activity of this species could be altered by the administration of penicillin to the dams during the last days of pregnancy and during lactation, and to determine if the same evidence of transmissibility could be observed in the rat as in the hamster. As in the hamster studies, the offspring of the penicillin-fed females developed very few, small carious lesions as long as they were not housed with caries-active rats. However, when the offspring of penicillin-fed females were housed with control rats of the same age, an increased level of caries initiation was observed. These

observations support the findings for hamsters and the hypothesis that the microorganisms responsible for dental caries are transmissible.

In other studies, Fitzgerald and Keyes (*J. Am. Dent. Assn.* **61,** *9 (1960)*) observed that the inoculation of caries-inactive hamsters with single or pooled cultures of five biochemically similar strains of streptococci isolated from a carious lesion in a hamster would induce the development of carious lesions. Pooled cultures of six strains of diphtheroid organisms or six strains of lactobacilli isolated from caries-active hamsters were ineffective. All of these streptococci were acidogenic, but none was proteolytic toward gelatin or milk. The strains of streptococci used had been "labelled" prior to inoculation by the induction of resistance to streptomycin, which made it possible to reisolate and identify these organisms. These microorganisms were found in the oral cavities and feces of hamsters that had not been inoculated but which were housed in the same cage with inoculated hamsters. A strain of streptococci capable of inducing dental caries in germ-free rats was inactive when tested in the hamsters, although it could be shown to have persisted in the oral cavity throughout the experimental period.

These data point out with great clarity the need to assure that the necessary oral flora for the production of carious lesions is present; otherwise, an inherently caries-susceptible strain of rodents fed a highly caries-producing diet for an adequate experimental period might not develop carious lesions. In all probability this has happened in repeated experiments and may explain some of the controversies prevalent in the experimental literature.

The above studies raised the question of whether any presently available strain of laboratory animals that had been developed on the basis of phenotypic selection could truly be termed inherently caries-resistant. The possibility existed that the selection of breeding animals on the basis of the absence of grossly detectable carious lesions would mean an inadequate source of cariogenic microbial flora for the contamination of the new generation. In other words, the offspring in the succeeding generations might be inherently caries susceptible but never be permitted to express their latent proneness to carious lesions in the presence of a cariogenic diet because of the absence of the appropriate flora.

In addition to raising the above question, the studies by Keyes (*loc. cit.*) provided the technics to seek the answer. S. Rosen, C. A. Hoppert and H. R. Hunt (*J. Dent. Res.* **39,** *721 (1960)*) employed a cross-fostering technic with Hunt-Hoppert caries-susceptible and caries-resistant rats. Entire litters of caries-susceptible and caries-resistant rats were exchanged within 24 hours of birth so that caries-susceptible mothers suckled caries-resistant litters and caries-resistant mothers suckled caries-susceptible litters. In both resistant and susceptible strains, the offspring had caries activities that reflected their genetic background and that did not suggest any influence of the foster mother.

More extensive studies have been conducted with the Harvard caries-susceptible and caries-resistant strains (J. H. Shaw and D. Griffiths, *Arch. Oral Biol.* **2,** *15 (1960)*). Weanling caries-resistant rats were housed with weanling caries-susceptible rats and weanling caries-resistant rats with young adult caries-susceptible rats that had developed numerous grossly visible carious lesions. In both trials the caries-resistant rats manifested very low caries activities until they were sacrificed at 250 days of age. In a second experiment, a cross-fostering experiment with the Harvard strain was undertaken in which the procedure differed from the study by Rosen *et al.* (*loc. cit.*) in that half litters were exchanged at birth. In this way the caries-susceptible mother nursed half of her own litter and half of a caries-resistant litter while the caries-resistant mother nursed half of her own litter and half of a caries-susceptible litter. Again the

offspring in these strains developed caries activities that were typical in every case of their genetic heritage and showed little or no influence of the foster mother.

These experiments with the Hunt-Hoppert and with the Harvard strains seemed to indicate that even when opportunities were provided for inoculation of the oral cavities of the caries-resistant strain with strongly cariogenic microorganisms from caries-active rats, there was a continued high resistance. Likewise, when caries-susceptible offspring were maintained from birth with a minimal contact with caries-active rats, the necessary oral flora was established readily and a high caries activity was demonstrated. The implication is that the caries-producing oral flora could be readily established in caries-susceptible rats maintained in an ordinary animal room and that the caries-resistant rats would remain resistant in the presence of an adequate inoculation.

Obviously these studies clearly indicate that the Hunt-Hoppert and Harvard strains have inherited traits tending toward high caries-susceptibility on the one hand and high caries-resistance on the other. What are the biochemical and nutritional parameters that enable the resistant rat to have a low caries activity in the presence of an adequate flora and a cariogenic diet and the susceptible rat to develop lesions so readily on the same diet? This question is challenging and its answer probably can be obtained only with rigorous studies where all potential variables of oral flora, cariogenic diet post-developmentally and developmental diet are controlled.

ATHEROSCLEROSIS IN MINIATURE PIGS

Tissue lipids were determined and atheroma histology was studied in miniature swine injected with C^{14} acetate in groups with saturated or unsaturated fat and with or without added cholesterol.

Many studies of the effects of varying diet fat composition have demonstrated numerous relationships between the nature of diet fat, levels of blood cholesterol or other blood lipids, and atherosclerosis. In fact the experiments of R. B. Alfin-Slater, L. Aftergood, A. F. Wells and H. J. Deuel, Jr. (*Arch. Biochem. Biophys.* **52,** *180 (1954)*) and Deuel *et al.* (*J. Nutrition* **55,** *337 (1955)*) showed that liver cholesterol increased when blood cholesterol decreased during the feeding of essential fatty acid-deficient diets indicating that reduced blood cholesterol may be only a component of cholesterol redistribution.

The recent study by R. Reiser, M. F. Sorrels and M. C. Williams (*Circulation Research* **7,** *833 (1959)*) was designed to determine the response of various tissues to low fat diets and to diets with high levels of saturated and unsaturated fat with and without added cholesterol. Labeled acetate incorporation was used to study the degree of cholesterol synthesis with various diets. Swine were used because of the similarity of their atheroma to those of man and because their size permitted individual analyses of coronary artery and aorta sections.

The "miniature swine" used in this study were divided in six groups with animals of a common heredity distributed evenly in each group. Three diets differing in fat content were fed with or without added cholesterol. The basic low-fat diet consisted of adequate vitamin, mineral and methionine supplements added to a mixture of nearly equal amounts of extracted soybean oil meal and sucrose. The unsaturated fat was cottonseed oil, and the saturated fat was a mixture of lauric and myristic triglycerides replacing 20 per cent of the sucrose.

Animals were weighed at the beginning

of the experiment and at monthly intervals. The amount eaten was recorded and fat intake was calculated. Feces were analyzed for total fatty acids and nonsaponifiable material.

After 11 months the surviving animals were injected with 1-C^{14} sodium acetate and killed 24 hours later. Tissue, plasma, and bile samples were removed and stored frozen until analysis. After extraction the fat was partitioned by silicic acid chromatography and the various fractions were analyzed.

Grading of atheromatosis in the low-fat group showed little evidence of lesions even with the addition of cholesterol to the ration. The myristic-lauric diet itself had no significant effect on atheromatosis until cholesterol was in the diet. Inclusion of cottonseed oil produced no lesions until cholesterol was added to the regimen. The numerous lesions produced by the saturated fat plus cholesterol stained poorly with Sudan IV and appeared to be fibrotic and calcified. In contrast the cottonseed oil plus cholesterol feeding produced plaques that were deeply stained with Sudan IV, were larger, fewer, and more concentrated near orifices of the aortic branches.

Post-prandial lipemia was not visible after any meals. Serum phospholipids were determined at intervals after ingestion of a saturated fat diet by animals previously on a low fat ration. Phospholipid levels rose above the starting value, then fell below the basal level during a nine-hour span. Feeding cottonseed oil resulted in a lowering of the phospholipid level with no return to the basal levels in nine hours.

Because of the premature death of many animals it was only possible to inject C^{14} acetate or take blood samples in about half of them. Most tissues were sampled but statistical evaluation was not feasible.

Total cholesterol levels increased in the absence of cholesterol in all tissues of all animals receiving fat-supplemented diets. The liver and plasma free and esterified cholesterol values were higher in the unsaturated fat groups. In the absence of dietary cholesterol the level of artery cholesterol was not influenced by the kind of diet fat.

Tissue cholesterol levels increased further with the addition of cholesterol to the diet. The feeding of unsaturated fat with cholesterol resulted in elevation of liver and coronary but not plasma cholesterol. Thus, doubt is raised as to the diagnostic value of the blood cholesterol level. In fact, these data suggest that unsaturated fat does not necessarily neutralize diet cholesterol. The effect of unsaturated fat may be solely due to its effect on absorption of dietary cholesterol. This value was 60 per cent on the saturated fat diet, 67 per cent on the low fat diet, and 87 per cent on the cottonseed oil diet. Reabsorption of biliary cholesterol would also be enhanced by this latter ration.

Triglyceride values were higher in the saturated-fat groups. Blood phospholipid changes paralleled the cholesterol changes.

Inspection of the relative specific activities of free and esterified cholesterol revealed a greater degree of incorporation of C^{14} in liver than in arteries. Blood cholesterol radioactivity measurements reflected the activity observed for liver cholesterol suggesting that blood cholesterol originated in the liver. Free cholesterol showed more activity than the ester form.

A high degree of synthesis of cholesterol was apparent in the animals fed a saturated fat, cholesterol-free diet. Addition of cholesterol produced a severe suppression of incorporation of labeled acetate into cholesterol. Correlation of the levels of blood and liver cholesterol was difficult due to the interaction of such variables as suppression of synthesis, dilution with endogenous cholesterol, and variation in the extent of absorption. Digitonin-precipitable sterols of the bile and bile acids showed a much greater specific radioactivity in the swine fed the saturated fat-rations without cholesterol than in those on any other diet.

SEX DIFFERENCES IN SUSCEPTIBILITY TO VITAMIN K DEFICIENCY

Male rats had a higher incidence of hemorrhagic deaths than females when maintained on either a synthetic vitamin K-deficient diet or on one containing irradiated beef as the protein.

V. C. Metta, M. S. Mameesh, and B. C. Johnson reported that feeding irradiated beef to growing male rats produced a hemorrhagic syndrome, apparently due to a vitamin K deficiency (*J. Nutrition* **69,** *18 (1959)*). They also noted that the female rat is not as susceptible to the vitamin K deficiency and the hemorrhagic syndrome as the male.

Recently, Metta and Johnson (*J. Nutrition* **72,** *455 (1960)*) carried out further studies in which they attempted to determine why the male rat is more susceptible than the female rat to vitamin K deficiency. There are several possible explanations for this phenomenon: the female may practice coprophagy to a greater extent than the male; or because of the greater food intake and thus increased body growth, the male may develop a vitamin deficiency more rapidly than the female; or there actually may be a sex difference dependent upon hormonal differences.

Metta and Johnson set up a series of studies in which rats were maintained on a diet deriving protein from a gamma ray irradiated beef (irradiated at 2.8 million rad). The beef made up 36 per cent of the diet; other constituents allowed adequate amounts of various nutrients. In the various groups studied, hemorrhagic death occurred in 78 of the 138 growing male rats (the first death occurring on the ninth day of study), but only in two of the 90 growing female rats (deaths occurring on day 188 and day 364).

As controls, 40 male and 40 female rats were fed on a diet containing non-irradiated beef and only three male rats died from hemorrhage; none of the females died. In the latter control series the males died on day 105, 130, and 300, as compared to much earlier deaths in the animals fed the irradiated beef. Thus from this series of studies, there is no doubt that the male animals are much more susceptible to the hemorrhagic syndrome when maintained on irradiated beef, whether or not this effect is due to a vitamin K deficiency.

A second series of studies were undertaken to determine the effect of food intake, rate of body gain, and coprophagy on the susceptibility of the animals to vitamin K deficiency, as noted by the hemorrhagic syndrome. Thirty male rats and 25 female rats were divided into five groups and all animals were fed irradiated beef diets.

One group of male animals was fed a restricted food intake equal to that consumed by a group of female rats. No attempt was made to prevent coprophagy in either group. Eighty per cent of the male rats died of hemorrhagic syndrome; none of the female rats died. It was noted that after 56 days of being on the diet the mean plasma prothrombin time was 36 (28–44) seconds for the males and 13 (12–16) seconds for the females.

In another group of male animals, restricted in their food intake so that the body weights were equal to that of the female group, 50 per cent died of hemorrhages. They had a mean plasma prothrombin time of 18 (16–21) seconds. In contrast, all male rats which were allowed to eat ad libitum died of hemorrhages by day 56. Females maintained on the same diet with ad libitum feedings with coprophagy prevented had mean plasma prothrombin times of 17 (14–19) seconds; none of these animals died of hemorrhage.

Thus it appears that the male animals, even when consuming quantities of foods comparable to that eaten by the females and growing at a rate comparable to the females, had a greater incidence of death due to hemorrhage than did the female rats.

It should be noted that one group of male rats had high plasma prothrombin times (average 36 seconds) and the other group had plasma prothrombin time as low as the female rats, nevertheless there was a high incidence of hemorrhagic deaths in both groups of male rats.

Female animals prevented from practicing coprophagy did not develop the hemorrhagic syndromes when maintained on this diet and the plasma prothrombin times, 17 (14–19) seconds, were not significantly different from those who were allowed to practice coprophagy.

These investigators then switched to synthetic vitamin K-deficient rations in which casein was the protein. Crystalline vitamins were added to the mixture with the omission of vitamin K. Four male rats and seven female rats were maintained on this diet in cages which would prevent coprophagy. Two of the male rats died from hemorrhagic syndrome on the twelfth day of feeding. On the fourteenth day of feeding the two remaining male rats had prothrombin times of 44 and 98 seconds. Two of the seven female rats maintained on the same regimen died from hemorrhage on day 16 and 69, respectively. No prothrombin determinations were carried out with this group. Thus it appears that under these conditions the female apparently is much more resistant to vitamin K deficiency.

Two additional groups, seven rats per group, were maintained on the vitamin K-free synthetic diet and, in addition, were given sulfathalidine to reduce the bacterial flora of the intestine and decrease the rate of the synthesis of vitamin K by the intestinal organisms. These animals were not maintained under conditions which would prevent coprophagy. They were studied for 26 days. Two of the male rats died on day 24; none of the female rats died. The mean prothrombin times of the plasma of the male rats was 47 (21–94) seconds while that of the female rats was 34 (15–96) seconds. The prothrombin times noted on this last group of female animals were comparable or even greater, at least in spread, than those noted for the male animals which were pair fed with female rats. Nevertheless, the male rats with average prothrombin times of 36 and 18 seconds showed 80 and 50 per cent deaths from hemorrhagic syndromes. Perhaps there are factors other than prothrombin involved in the deaths of the animals.

These investigators have shown that the male animal apparently is more sensitive to vitamin K deficiency than the female rat and that these effects are not dependent on the rate of growth, food consumption, or whether or not the animal practices coprophagy, but may be related to the female sex hormones. In the present study, however, there is nothing to substantiate this hypothesis.

ZINC DEFICIENCY IN CHICKS

A zinc deficiency develops in chicks fed rations containing soybean or sesame meal as the source of protein. The deficiency occurs even though there is an adequate level of the metal in the ration.

A few years ago E. J. Underwood (*Trace Elements in Human and Animal Nutrition, Academic Press, New York (1956)*) stated that "There do not appear to have been any attempts to induce zinc deficiency in pigs or poultry by the use of specially purified diets. A deficiency of this element under any reasonable practical conditions seems extremely unlikely because the cereal grains and their by-products, which form the basis of most rations for these species, contain liberal amounts of zinc." The ink was scarcely dry on the pages of this book when reports appeared of parakeratosis among swine fed practical rations. This condition appeared shortly after the level of calcium

in the rations was increased (*Nutrition Reviews* **15,** *334 (1957)*).

The deficiency syndrome in the swine was followed almost immediately by reports of a similar deficiency in chicks. A thorough study of this condition was made by A. B. Morrison and H. P. Sarett (*J. Nutrition* **65,** *267 (1958)*). They maintained day-old chicks on the basal diet for the first twelve days of life. At the end of that time they were separated into groups.

The ten chicks which were maintained on the basal soybean diet (Drakcett Assay Protein) gained 195 g. during the 14-day experimental period. Those receiving the same diet plus 5 p.p.m. of added zinc gained 234 g. during this period. Higher levels of zinc produced no further increase in weight gains.

The increased weight gain resulting from the added zinc was surprising since previous attempts to produce a zinc deficiency in experimental animals required diets and environments that were specially designed to make them extremely low in this metal. The above study differed from the earlier work in that (1) the basal diet contained 30 p.p.m. of zinc; (2) the chicks were the progeny of hens fed commercial diets; and (3) they were housed in galvanized (zinc-coated iron) brooders and received tap water in galvanized pans.

In another study, additional calcium was added to the soybean diet. This supplement reduced the two-week weight gain from 188 g. for the basal group to 161 and 152 g. for the 0.5 and 1.0 per cent calcium-supplemented groups, respectively. The addition of 5 p.p.m. of zinc to the basal diet increased the weight gain from 188 to 218 g. The same amount of zinc added to the calcium-supplemented diets had little effect on weight gain. The addition of 25 p.p.m. of zinc was effective in overcoming the growth inhibition of the calcium.

Eight of the 19 chicks receiving the soybean basal diet showed a malformation of the tibiotarsal-tarsometatarsal joint (hock), characterized by enlargement and elongation of the joint, but no change in the percentage of bone ash. The addition of 1.0 per cent calcium to the diet increased the incidence of joint abnormalities to 13 of 20 chicks. The addition of zinc decreased the incidence, and in the group receiving 25 p.p.m. of zinc none of the chicks showed any bone abnormality. The length of the tibiotarsal bones of the chicks on the soybean diet averaged 52 to 55 mm. while the chicks receiving adequate zinc had bones 59 to 60 mm. long. There is a possibility that the shortening of the bones is a reflection of the reduced body weight rather than of the zinc deficiency per se, since there appears to be a correlation between bone length and the weight of the chicks at the end of the experiment.

The chicks fed a similar diet, with the exception that the protein source was casein and gelatin, showed no improvement in growth when zinc was added. This occurred even though the casein-gelatin basal diet contain 30 p.p.m. of zinc (exactly the same as the basal soybean diet). The chicks on the basal casein-gelatin diet gained 219 g. during the two-week period, which was as good or better than the gain secured on the zinc-supplemented soybean diet. Furthermore, the addition of 1 per cent calcium to the casein-gelatin diet did not depress growth.

The assumption that supplemental calcium interferes with the absorption of zinc appears to be true only for rations that contain plant proteins. Calcium did not interfere with the absorption of zinc when the protein in the diet was a mixture of casein and gelatin.

Morrison and Sarett reported that the zinc deficiency in their chicks was not associated with any reduction in fasting blood sugar level, or serum pyruvic acid, lactic acid, and alkaline phosphatase levels, but it did result in an increased intestinal level of alkaline phosphatase.

More recently, J. G. Lease and co-workers observed (*J. Nutrition* **72,** *66 (1960)*) that when chicks were fed rations containing

sesame meal they developed symptoms resembling those reported for zinc deficiency. This occurred even though the birds were raised in galvanized cages, received tap water in galvanized pans, and secured added zinc from the salt mixture. The chicks showed a reluctance to move about, exhibited marked leg deformities, shortened shanks, poor growth, and in some experiments, frizzled feathers.

When these chicks were fed a semi-purified diet containing 31 to 35 per cent of sesame meal, they weighed 300 g. at the end of four weeks. A large percentage of the birds showed bone abnormalities. The addition of 30 p.p.m. of zinc to this diet increased the body weight to 349 g., while 60 p.p.m. increased it to 364 g. and practically eliminated the bone abnormality. When analyzed, the basal diet contained 29 p.p.m. of zinc.

The following evidenced an organic substance in the sesame meal which made the zinc unavailable to the chick: (1) autoclaving the sesame meal produced body weight gains comparable to that seen when 30 p.p.m. of zinc were added; (2) the addition of EDTA (ethylenediaminetetracetic acid, disodium salt) released zinc from the metal-complexing substances in the sesame meal, or it was able to destroy the zinc-binding power of the sesame meal so that the extraneous zinc became available to the chick.

The above studies indicate that certain proteins may increase the animal's requirement for zinc, presumably by making the zinc in the ration unavailable. In the case of the sesame meal, the evidence provided by Lease *et al.* suggests that this source of protein is able to make unavailable even a certain amount of added zinc. These results further indicate that the effect of added calcium on the absorption of zinc is seen only in the presence of certain proteins (probably of plant origin), but not when others are present.

The inability of the chick to use normal amounts of zinc when soybean or sesame meal are present in the ration raises the question as to whether a similar situation exists in the unprocessed seeds. If the "binding" of the zinc occurs when the unprocessed seeds are used in the chick rations, one might wonder about the role played by the "binding" substance in the physiology of the plant.

The work with zinc emphasizes the fact that an analysis of a ration for a metal does not necessarily provide any indication as to whether the metal will be available to the animal. Just as bipyridyl provided a rough index of the availability of iron, it is hoped that similar procedures will soon be developed for the other elements.

DIETARY PROTEIN AND ACETATE-1-C^{14} INCORPORATION

A low dietary protein level tended to increase at least initial incorporation of acetate-1-C^{14} into liver cholesterol in chicks and also increased the rate of fatty acid biosynthesis.

It has been shown that a high-protein diet tends to depress the atherogenic effect of dietary cholesterol and fat in chicks (M. Kokatnur, N. T. Rand, F. A. Kummerow and H. M. Scott, *J. Nutrition* **64,** *177 (1958)*). However, it is not clear whether this influence of protein may not, in part, have been due to its lipotropic effect, since the high serum lipid and cholesterol levels in chicks that had been fed a low-protein diet deficient in methionine and choline was lowered by the simple addition of more protein (T. Nishida, F. Takenaka and Kummerow, *Circulation Research* **6,** *194 (1958)*).

Other workers who have noted such an effect of dietary protein on serum cholesterol levels include J. Stamler *et al.* (*Circulation Research* **6,** *442 (1958)*) and D. Johnson *et al.*

(*J. Nutrition* **66,** *367 (1958)*). These investigators were also uncertain as to whether the effect was partially lipotropic and to what extent active synthesis of lipid was involved.

Nishida, A. Ueno and Kummerow (*J. Nutrition* **71,** *379 (1960)*) reason that serum cholesterol levels may be considered representative of a dynamic equilibrium between the rate of introduction and the rate of removal of cholesterol from the blood. Since the liver is the main site of both biosynthesis and degradation of cholesterol, the hypocholesteremic effect of dietary protein could be partially attributed to a decrease in biosynthesis of cholesterol, or to an increase in degradation of cholesterol to bile acids in the livers of birds fed a high-protein diet as compared with those fed a low-protein diet. They therefore designed a study to observe the effect of dietary protein on the *in vivo* incorporation of acetate-1-C^{14} into liver and serum cholesterol and fatty acids, liver protein, and liver glycogen in the presence or absence of dietary cholesterol.

Sixty New Hampshire-Columbian cross chicks were divided into four groups of 15 birds each and for three weeks fed diets which contained 15 or 30 per cent of protein, with and without 1 per cent of dietary cholesterol. The chicks were then injected intraperitoneally with sodium acetate-1-C^{14} and bled from the heart at 15, 30, 45, 60, and 120 minutes after injection. The livers were immediately frozen in liquid nitrogen and stored at $-20°C$.

A higher initial incorporation of acetate-1-C^{14} into liver cholesterol occurred in chicks that had been fed a low-protein diet than in those fed a high-protein diet. Furthermore, the presence of dietary cholesterol depressed the percentage of incorporation of acetate-1-C^{14} into liver cholesterol. (Such marked inhibition of the *in vivo* and *in vitro* biosynthesis of cholesterol has been reported in several species of animals fed dietary cholesterol (R. G. Gould *et al.*, *J. Biol. Chem.* **201,** *519 (1953)*; G. M. Tomkins *et al.*, Ibid. **201,** *(1953)*; and R. G. Landgon and K. Bloch, Ibid. **202,** *77 (1953)*).

The liver cholesterol concentration in birds consuming diets containing 30 or 15 per cent of protein increased 3.5- or 7-fold respectively when 1 per cent of cholesterol was added to the diets, indicating that the cholesterol-elevating effect was more pronounced at the low-protein level than at the high-protein level. However, in the presence of dietary cholesterol, the percentage of incorporation of acetate-1-C^{14} into liver cholesterol in chicks fed a low-protein diet was greater than in those receiving a high-protein diet. When dietary cholesterol was withheld, the level of liver cholesterol of chicks in both groups was the same but the birds on the low-protein diet incorporated more acetate-1-C^{14} into liver cholesterol.

The initial uptake of acetate-1-C^{14} into serum cholesterol was also higher in birds on the low-protein diet in spite of a significantly higher serum cholesterol level.

A low-protein diet resulted in a significant elevation of the total liver fatty acids and also increased the incorporation of acetate-1-C^{14} into liver fatty acids. Whereas dietary cholesterol had depressed the rate of cholesterol biosynthesis, it stimulated the rate of synthesis of fatty acids in the liver; this was reflected by a higher activity in the fatty acids of serum.

In the presence of dietary cholesterol, no significant difference in incorporation ratios (fatty acids vs. cholesterol) was observed between chicks kept on low-protein diets and those maintained on high-protein regimens. In each of the above cases, however, the incorporation ratios were increased because of the elevating effect of dietary cholesterol on fatty acid biosynthesis and its depressing effect on the synthesis of cholesterol.

The level of protein in the liver was significantly higher in animals given high-protein diets than in those fed low-protein diets. Feeding of cholesterol also decreased the proportion of protein in the liver. However, the percentage of uptake of acetate-1-C^{14}

into liver protein was not affected by these dietary factors. The glycogen content of the liver was unaltered by dietary protein and cholesterol.

The authors point out that the effect of a low-protein diet in stimulating cholesterol biosynthesis was demonstrated even when cholesterol concentration in the liver was high. Under these conditions the stimulation was very much less than that observed when the liver cholesterol level was low. Even so, it appears that cholesterol biosynthesis in the liver is influenced by factors other than liver cholesterol concentration (postulated by many investigators who envisage a close homeostatic regulation by dietary cholesterol).

These results also indicated that a close relationship exists between cholesterol biosynthesis and fatty acid biosynthesis. An increase in the production of cholesterol was accompanied by an increase in fatty acid uptake of the radioactive label; dietary cholesterol also stimulated fatty acid biosynthesis.

In this study the level of choline was below the optimum for chicks (although the liver lipid content in the chicks did not indicate a significant deficiency in lipotropic factors). The authors point out, however, that the beneficial effect of a high-protein diet on cholesterol metabolism cannot be explained fully by the presence of sufficient methionine. The elevating effect of a low-protein diet on the serum cholesterol level in chicks may be due to a combination of factors such as shortage of lipotropic materials, pronounced lipogenesis, and possibly cholesterol catabolism.

FATTY ACID UPTAKE BY SKELETAL MUSCLE

Free fatty acids are taken up by electrically stimulated skeletal muscle in the anesthetized dog.

B. Issekutz, Jr. and J. J. Spitzer (*Proc. Soc. Exp. Biol. Med.* **105,** *21 (1960)*) have further substantiated evidence that free fatty acids may be used directly by skeletal muscle by determining free fatty acid uptake in the thigh muscles of dogs. They measured blood flow from the profunda femoris veins draining the thigh muscles in 11 dogs under barbiturate anesthesia. Free fatty acids were determined in the vein and femoral artery. The uptake was calculated from the flow and arteriovenous differences before and after direct electrical stimulation of the thigh muscles.

The fatty acid concentration was higher in the vein than in the artery. Upon electrical stimulation of the thigh muscles blood flow through the profunda femoris veins increased as much as 16-fold; the free fatty acid concentration in profunda femoris veins fell below that in the femoral artery in 14 of 15 experiments on 11 dogs. Control arteriovenous differences "on the intact, nonstimulated side showed no consistent change during stimulation."

Results are shown for one "typical" experiment. A negative arteriovenous difference (0.050 microequivalents per ml.) became slightly positive after stimulation; at the same time blood flow increased 16-fold. After a second stimulation the arteriovenous difference was more positive (up to 0.050 μE. per ml.) and blood flow increased sevenfold. The calculated uptake of free fatty acid in this experiment was minus two to minus four μE. per minute before stimulation and plus six to eight μE. per minute after stimulation.

Issekutz and Spitzer recognize that the lack of fatty acid uptake at rest might have been due to anesthesia. Unfortunately, data are given for only the one "typical"

experiment; it would have been desirable to tabulate the results of the other 14 experiments.

It was not practical for the authors to control the effects of insulin, epinephrine and glucose, all powerfully effective in altering fatty acid uptake and release. One wonders how changing level of these substances, induced by anesthesia, operation and muscle stimulation, affected the result. Blood from the subcutaneous adipose tissue, a large source of free fatty acids, could not be excluded from the experimental preparation. Though ligation of the femoral vein below the entrance of the profunda femoris was intended to lessen it, one must assume that a fair amount of blood from the subcutaneous tissue found its way into the deep veins.

Nevertheless, evidence exists that cardiac muscle takes up fatty acid directly (*Nutrition Reviews* **16,** *102 (1958)*). In spite of the uncontrolled factors, it appears that the authors have demonstrated that in the dog stimulated skeletal muscle takes up free fatty acid.

ANTIBODY FORMATION BY VITAMIN DEFICIENT RATS

Rats deficient in pantothenic acid or pyridoxine were unable to make antibodies in a normal fashion in response to an influenzal vaccine. Deficiency of thiamine did not inhibit antibody production.

For a number of years A. E. Axelrod and his associates have been studying the ability of deficient animals to respond to antigenic stimulation. Much of their work was reviewed in this journal (*Nutrition Reviews* **10,** *353 (1950)*). Others have confirmed the fact that deficiencies of pantothenic acid or of pyridoxine impair the ability of animals to withstand infection and inhibit their capacity to produce antibodies (Ibid. **15,** *47 (1957)*). Although Axelrod's work has been carefully controlled by pair feeding, one criticism which has been offered in the past has been that he has limited his antigenic stimulus to that produced by human erythrocytes or by diphtheria toxoid. Other investigators, employing viruses as antigens, reported failure to demonstrate impairment of antibody production by a deficiency of pyridoxine (G. S. Mirick and W. B. Leftwich, *J. Exp. Med.* **89,** *155, 175 (1949)*).

In an extension of their previous studies Axelrod and S. Hopper (*J. Nutrition* **72,** *325 (1960)*) reported extensive observations in rats made deficient in pantothenic acid, pyridoxine, or thiamine. They employed standard dietary techniques both for their control animals and for the deficient groups. The antigen used was a virus preparation of the influenza strain PR-8. Injections of this antigen were given intraperitoneally once weekly beginning with the fifth week of the deficiency for three consecutive doses. One week following the final dose the animals were bled and serum was measured for its content of antibody employing the hemagglutination inhibition technique.

Pantothenic acid-deficient animals were found to have marked inhibition of their ability to form antibodies when compared with control animals in this group were not pair fed. Pyridoxine-deficient animals likewise had inhibition of their antibody production, and in one portion of this study the controls were pair fed. It was found that inanition of the controls did not impair their ability to produce antibodies. In a third study rats deficient in thiamine formed antibodies as well as the control animals.

This most recent report by Axelrod's group sustains his previous observations that antibody production is dependent upon certain essential nutrients in the diet

and that this observed inhibition of antibody production is not a general one depending upon anorexia and inanition. The observation that antibody production in response to a viral antigen is inhibited in a similar fashion to that of two other types of antigen seems to indicate that a deficiency of pantothenic acid or of pyridoxine induces an immunologic paralysis.

This study of the relation of dietary essentials to immune mechanisms represents another step in the prolonged search for an understanding of immunological mechanisms.

LINOLEIC ESTER AND CHOLESTEROL

Weanling rats were fed either high or low fat diets containing cholesterol supplemented with ethyl linoleate. This ester reduces carcass and plasma cholesterol levels.

Several experiments have been performed recently to determine the nutritional effects of single unsaturated fatty acids. Such studies include the review by I. H. Page *et al.* (*Circulation* **16,** *163* (*1957*)) and the experiments of J. T. Anderson, A. Keys, and F. Grande (*J. Nutrition* **62,** *421* (*1957*)) in which diets of controlled fat content were fed to adult male human subjects. The fats studied included butterfat, olive oil, cottonseed oil, corn oil, sunflower seed oil, coconut oil, sardine oil, and "house diet" fat. They observed that the serum cholesterol response correlated roughly with the principle that saturated fat diets led to higher cholesterol levels than polyunsaturated fat diets, but that neither degree of unsaturation nor content of linoleic acid explained the results.

In the study reported by D. M. Hegsted, A. Gotsis and F. J. Stare (*J. Nutrition* **63,** *377* (*1957*)) a large number of oils were tested in cholesterol-cholic acid-fed rats. They observed that "non-essential" unsaturated fatty acids promoted hypercholesteremia which was countereacted either by essential fatty acids or saturated fatty acids.

In only a few cases have experiments been reported testing the effects of individual fatty acids on cholesterol levels. The authors of this study have used a highly purified preparation of ethyl linoleate in order to circumvent the problem of interpretation arising from using fat preparations of unknown or complex composition (F. W. Quackenbush and M. D. Pawlowski *J. Nutrition* **72,** *196* (*1960*)).

Using fractional crystallization of urea addition compounds followed by low pressure distillation the authors prepared a *cis* 9, 12 linoleic ester at least 97% pure. The diets were either 20 per cent or 2 per cent fat which was either hydrogenated coconut oil (HCO) or soybean oil (SBO). Cholesterol was 1 per cent and sodium glycocholate was 0.5 per cent of the diet. Casein was 18 per cent of the diet.

Weanling male and female rats were used. The first three groups had 12 males and 12 females in each group and there were eight males each in groups four, five, and six.

Group one was fed the 20 per cent soybean oil diet. Group two receiving a 20 per cent hydrogenated coconut oil diet was divided at the end of five weeks so that half the animals of each sex were fed 10 per cent of HCO and 10 per cent of linoleic ester. The third group (2 per cent HCO) was divided at the end of five weeks so that half the animals of each sex were given 2 per cent linoleic ester instead of HCO. Groups four, five, and six were fed 2 per cent HCO through five weeks. Group four was then given 2 per cent SBO and group five was given 4 per cent SBO. Group six received a 25 mg. supplement of linoleic ester daily to the 2 per cent HCO diet.

Plasma was analyzed for free and total cholesterol, and liver and carcass were analyzed for total lipid and cholesterol.

Animals receiving the hydrogenated coconut oil diets gained substantially less weight than those getting the soybean oil ration, but later replacement of half the HCO with linoleic ester improved the growth. Essential fatty acid deficiency evidenced by scaliness of paws and tails appeared by the third week on the HCO diet and continued through the experiment, being more pronounced in males.

Plasma cholesterol levels averaged about 50 mg. per 100 ml. in weanling rats with about 80 per 100 ml. as ester cholesterol. In some cases the animals getting high or low fat HCO diet for four weeks had total plasma cholesterol values as high as 1000 mg. per 100 ml. in contrast to soybean oil animals averaging about 200 mg. per 100 ml.

The plasma cholesterol levels were lowered as soon as linoleate was introduced. For example all the plasma total cholesterol values for the males receiving 2 per cent HCO were above 200 mg. per 100 ml. ranging from 200 to 900 mg. and all values for linoleate substituted groups were below 200 mg. per 100 ml. Similar responses were observed whether all or part of the HCO was replaced by linoleate. Males in the soybean oil groups had slightly higher plasma cholesterol values than those eating the 2 per cent linoleate ration. The level of fat in the diet did not markedly influence the cholesterol level, in agreement with the observations of N. Nath, R. Wiener, A. E. Harper, and C. A. Elvehjem (*J. Nutrition* **67,** *289 (1959)*).

Although the cholesterol responses of the female rats paralleled those of the males, the plasma cholesterol levels were significantly higher in all cases.

Variation within diet groups was marked especially when cholesterol levels were high. For this reason some blood samples were taken from fasted animals in order to determine whether fasting would improve the agreement between values. No lessening of variance was significant except in the soybean groups. In the animals fed hydrogenated coconut oil the cholesterol levels were significantly higher in fasted than in non-fasted animals.

Livers of animals fed the high fat diets stored from 50 to 80 per cent of the total body cholesterol. Animals on the 2 per cent HCO diet also had similarly high liver cholesterol. Animals on the 2 per cent linoleate regimen were in sharp contrast with only 13 to 19 per cent of total body cholesterol present in the liver and with greatly reduced total carcass stores. When the linoleate was reduced to the minimum needed to prevent symptoms of essential fatty acid deficiency (25 mg. per day), it was insufficient to reduce liver or total carcass cholesterol on the 2 per cent HCO diet.

The experiments in this report indicate that the ester of linoleic acid of relatively high purity is effective in reducing liver and total carcass cholesterol as well as plasma cholesterol in the rat.

KWASHIORKOR AND PLANT PROTEINS

Pathological lesions observed in rats force fed certain plant foodstuffs resemble some of the alterations found in kwashiorkor, a disease of protein-malnourished infants.

H. Sidransky and E. Farber (*Arch. Path.* **66,** *119 (1958)*; **66,** *135 (1958)*) have produced pathological lesions in young rats resembling many of the alterations described in kwashiorkor (*Nutrition Reviews* **15,** *101, 132, 169, 199, 207, 254, 264 (1957)*; **16,** *36 (1958)*; **17,** *259 (1959)*) by force feeding them from three to seven days with purified

diets devoid of certain single essential amino acids. These results have raised the question of whether similar changes might be induced by force feeding diets in which the sole source of protein is natural plant foodstuffs known to be deficient in certain amino acids.

In a series of experiments designed to test this concept, Sidransky (*J. Nutrition* **71,** *387 (1960)*) composed five diets consisting of either corn, rice, wheat, cassava or milo as the sole source of protein. These were force fed to young rats for three days or fed ad libitum for seven days. Morphological changes in organs of rats thus fed were studied and selected biochemical determinations made.

The vitamin-sucrose mixture added to the plant flour furnished the following number of mg. of vitamins to each 100 g. of diet: thiamine, 0.25; Ca pantothenate, 2.0; niacin, 1.0; choline chloride, 100.0; biotin, 0.01; folacin, 0.1; inositol, 10.0; menadione, 0.1; cyanocobalamin (vitamin B_{12}), 0.01; and cod liver oil. Each ration was blended with water so that the resulting mixture could be administered by stomach tube. Feedings were given daily at 9 A.M., 3 P.M., and 9 P.M. for three days. The rats received 6 g. on the first day, 8 g. on the second day, and 10 g. on the third day. On the fourth day the rats were exsanguinated, approximately 14 hours after the last feeding. Control animals received a commercial chow ad libitum and were fasted for 14 hours before sacrificed.

Rats on the ad libitum experimental diets were fasted overnight before initiation of the special diets for seven days. (Animals receiving the ad libitum diets for only four days displayed insignificant morphological and biochemical changes.) Whole body weights were obtained at the beginning and the end of each experiment. Organs were weighed fresh, using only the right organs of paired organs.

Rats force fed the corn diet gained the most weight, 7.1 g. in three days. Animals fed wheat, rice, or milo gained less, and animals force fed the cassava diet lost 0.8 g. of body weight over a three day period. The livers of animals fed corn, rice, wheat or milo diets weighed more than those of rats fed cassava or the control diet. The submaxillary and the spleen were frequently smaller in force-fed rats. In some experimental groups, the kidneys and gastrocnemius muscles were significantly smaller than those in controls. Increases of liver lipid (largely neutral fat) and glycogen was seen in the force-fed groups. All animals in these groups, with the exception of that group which received milo, displayed significant decreases in total liver protein.

Morphologically, the livers of force-fed rats appeared glistening and yellow-brown. After hematoxylin and eosin staining the cytoplasms were vacuolated predominantly in the periportal areas. With oil red O and Bests's carmine, the liver cells showed considerable amounts of both lipid and glycogen. When the livers were graded according to the amount of lipid observed histologically, the order from most to least lipid was corn, rice, cassava, wheat, and milo. The pancreas of rats fed the corn diet showed mild to moderate decreases in the cytoplasmic/nuclear ratios in the acinar cells. Approximately half the rats receiving rice, wheat, and cassava diets revealed similar atrophic changes. In rats fed corn, rice, cassava, or wheat, the submaxillary glands revealed a marked loss of cytoplasm in the acinar cells. Spleens of all force-fed rats showed atrophic changes, but no gross or microscopic changes were detected in hearts, lungs, kidneys, stomachs, small intestines or muscles.

Because no food was taken for 14 hours before sacrifice in the case of control rats, whereas the experimental animals were force fed 3.4 g. of diet 14 hours before sacrifice, a control experiment was performed in which rats fed the stock diet ad libitum were tube fed a sucrose solution (isocaloric with the 3.4 g. of experimental diet) 14

hours before sacrifice. However, there were no differences from the normally treated control rats.

In one experiment, rats were force fed the corn diet supplemented with 1.0 per cent of L-lysine and 0.5 per cent of L-tryptophan. Changes in the liver and other organs were identical with changes observed in animals fed the unsupplemented corn diet but over-all growth was increased.

Those rats receiving the experimental diets ad libitum for seven days ate less than controls and gained less weight. Although controls ate 9 g. of food per rat per day, those receiving experimental diets ate only the following amounts in grams per rat per day: corn, 5.3; rice, 6.1; wheat, 5.5; cassava, 4.9; and milo, 5.8. The rats fed the rice diet gained the most weight whereas those receiving cassava lost weight. Weights of livers, submaxillary glands, kidneys, gastrocnemius muscles and spleens were less in all experimental groups. Liver protein in all experimental groups was decreased as compared to controls; the liver lipid was decreased in some groups and the liver glycogen was increased. The only morphological changes noted were in the livers of occasional animals in each of the experimental groups. These showed mild periportal lipid accumulation. The greater the amount of corn or rice diet consumed, either by force feeding or ad libitum, the greater the liver weight which was largely due to increased liver lipid. Rats eating very little food showed minimal, if any, pathological changes.

The author relates the difference in pathology between the force-fed and ad libitum experimental groups to the smaller quantity of the deficient diet consumed by the rats eating by their own selection. He also divides the changes seen in organs after the experimental diets into two types: (1) those dependent on the quantity of diet consumed and (2) those due to deficient protein. The loss of organ weight is an example of the former whereas abnormal periportal deposition of fat is specific to the latter. The author points out that conditions precipitating kwashiorkor in the human infant are similar to the experimental conditions in force-fed rats, and that some of the pathological lesions and chemical changes observed in the rats force fed the above plant foodstuffs resemble some of the alterations found in kwashiorkor. Often, when this disease develops, the mother has fed the recently weaned infant or young child as much food as possible, even forcibly, but this food has been of a protein-deficient type.

PYRIDOXINE AND TRANSAMINASES

Deficiency of pyridoxine results in decreases of plasma alanine- and aspartic transaminases; the alanine enzyme appears to be the more sensitive.

Numerous investigators have shown that tissue transaminase activity is depressed in pyridoxine-deficient experimental animals (M. Brin and R. E. Olson, *Fed. Proc.* **10,** *166 (1951)*; Brin *et al.*, *J. Biol. Chem.* **210,** *435 (1954)*; E. F. Caldwell and E. W. McHenry, *Arch. Biochem. Biophys.* **45,** *97 (1953)*; F. Schlenk and A. Fisher, *Arch. Biochem.* **12,** *69 (1947)*). Although most of the early work was performed on the enzyme which involves aspartic acid, in several cases the enzymes capable of acting on alanine were found to be more markedly depressed than the aspartic system (Brin *et al.*, *loc. cit.*). Alanine transaminase is also markedly affected by X-irradiation *in vivo* by fasting and by administration of cortisone.

Brin, M. Tai, A. S. Ostashever and H. Kalinsky (*J. Nutrition* **71,** *416 (1960)*) have

attempted to ascertain whether serum transaminases are affected in the same manner by pyridoxine deficiency as the tissue transaminases. Earlier studies with monkeys (E. M. Marsh *et al.*, Ibid. **56,** *115 (1955)*) and humans (M. Sass and G. T. Murphy, *Am. J. Clin. Nutrition* **6,** *12 (1958)*) gave positive but generally variable results. Brin *et al.* therefore decided to use pyridoxine-deficient rats for their studies.

These albino rats were raised in wire-bottom cages and fed purified diets and water ad libitum. Pyridoxine was omitted from the basic formula in the deficient diets. Blood was drawn by cardiac puncture into heparinized syringes. The authors state that obtaining the blood sample by decapitation resulted in highly variable transaminase values, probably because of release of enzymes from the excised tissues.

A colorimetric technique was employed to determine the transaminase levels in which the pyruvate formed in the reaction of the enzyme with the amino acid substrate was isolated from the acid-treated incubation mixture as the phenylhydrazone. The color was developed with alcoholic KOH and read in a photoelectric colorimeter at 490 mμ (F. Wroblewski and P. Caubaud, *Am. J. Clin. Path.* **27,** *235 (1957)*). Brin *et al.* claim that this is an improvement over their previously employed assays.

In terms of total growth, the pyridoxine-deficient animals displayed the typical marked depression within the first week of the deficiency. Other obvious physical symptoms such as tremor were not evident until about three weeks had passed.

In the plasma of control rats the aspartic enzyme was found to be normally more active than the alanine enzyme. Addition of pyridoxal phosphate to the control plasma did not result in increased activity of either enzyme.

Activity of the alanine transaminase in the pyridoxine-deficient group was depressed by as much as 85 per cent. Addition of pyridoxal phosphate to such plasmas increased the enzyme activity to a considerable degree but not to normal values.

Supplementation did not stimulate the aspartic transaminase where activity had been depressed about 63 per cent by the deficiency of pyridoxine.

Because of these results the authors decided to determine what effect might be elicited by repletion of the intact animals with the limiting vitamin. Supplementation of deficient rats by injection of pyridoxine resulted in stimulation of the alanine enzyme to 80 per cent of the control value and the aspartic enzyme to 90 per cent of normal. After feeding the deficient diet for a period of two to four weeks, transfer to the control diet restored the activity of the alanine transaminase to normal and the aspartic enzyme returned to 75 per cent of the original value. The addition of pyridoxine *in vitro* had no additional stimulating effect on any of the enzymes obtained from rats treated as above.

Plasma transaminases were found to be depressed in pyridoxine-deficient adult rats as rapidly as in younger rats, but not to the same extent. As in the younger rats, the alanine transaminase was affected more markedly than the aspartic enzyme. In contrast to the result in young rats, addition of pyridoxine *in vitro* had no marked stimulatory effect on the alanine enzyme in the plasmas from deficient adult rats.

The authors point out that since the addition of pyridoxine to the deficient plasma resulted in enhanced activity of the alanine enzyme (in young rats), the transaminase effect was due to inadequate coenzyme relative to available apoenzyme in the plasma. The fact that pyridoxine did not result in complete restoration indicated that in pyridoxine deficiency, the apoenzyme production was also probably curtailed.

The differential effect seen between the extent of depression of the alanine transaminase versus the aspartic transaminase

provides evidence that these enzymes are two different proteins. The alanine enzyme may be the more sensitive test for the pyridoxine status of the animal.

VITAMIN B_6 AND B_{12} RELATIONSHIPS

Feeding rats a diet deficient in Vitamin B_6 results in the lowering of vitamin B_{12} reserve as manifested by a low B_{12} serum level and subnormal hepatic storage.

J. M. Hsu and B. F. Chow (*Arch. Biochem. Biophys.* **72,** *322 (1957)*) have demonstrated that vitamin B_6 deficiency brings about a partial impairment of the absorption of vitamin B_{12} by adult rats. Thus it would be expected that prolonged vitamin B_6 deficiency in rats would result in the development of vitamin B_{12} deficiency. B. Ranke, E. Ranke, and Chow (*J. Nutrition* **71,** *411 (1960)*) have obtained such data on the effect of prolonged feeding of a vitamin B_6-deficient diet on the vitamin B_{12} reserves of the liver and plasma B_{12}. They have also investigated the effect of vitamin B_{12} deficiency on serum concentrations of transaminase, an enzyme believed to be related to vitamin B_6 concentration.

Vitamin B_6 deficiency was induced by offering ad libitum to male weanling rats a vitamin B_6-deficient diet consisting of casein, sucrose, minerals and all known vitamins except vitamin B_6 (P. G. Tulpule and J. N. Williams, Jr., *J. Nutrition* **57,** *529 (1955)*). The rats were housed in screen bottom cages. Control littermates were supplied vitamin B_6 daily by injection of pyridoxine hydrochloride (50μg) and by supplementation of a diet otherwise identical to the one indicated above with pyridoxine (100 μg/kg.). The rats were kept on these regimens for six months, at which time the usual symptoms of acrodynia, loss of weight, and loss of appetite developed in the vitamin B_6-deficient group.

Preparation of animals with vitamin B_{12} deficiency was accomplished by selecting the male and female progeny of rats fed a soybean diet (C. T. Ling and Chow, *J. Biol. Chem.* **198,** *439 (1952)*) low in vitamin B_{12} content during pregnancy and lactation and offering those progeny the same soybean diet for six to eight months. For comparison, littermates were fed the same basal diet supplemented with vitamin B_{12} (100 μg/kg. of diet).

Vitamin B_{12} concentrations in sera or plasma were determined by the procedures of G. W. Gaffney *et al.* (*J. Gerontol.* **12,** *32 (1957)*). Livers were homogenized, treated with potassium cyanide, and assayed microbiologically for vitamin B_{12} activity.

To confirm the effect of vitamin B_6 deficiency on vitamin B_{12} absorption, radioactive vitamin B_{12} (Co^{60}) was given by mouth to vitamin B_6-deficient and vitamin B_6-injected animals. Deficient animals absorbed 32 per cent of the administered dose whereas treated rats absorbed 64 per cent of the vitamin B_{12}.

When vitamin B_6 was withheld for a three month period, the level of vitamin B_{12} in the serum was depressed to less than 15 per cent of the normal value. The amount of vitamin B_{12} in the liver of B_6-deficient animals averaged only a third of the normal value. Supplementation with vitamin B_{12} increased the level in the serums to slightly more than half of the control level, and the vitamin B_{12} in the liver was elevated to about two-thirds normal, showing that the ability of these animals to absorb vitamin B_{12} was not completely destroyed. Curiously, the hepatic level of vitamin B_{12} was considerably reduced when an extra large amount of vitamin B_6 was incorporated in the diet.

Animals deficient in vitamin B_{12} displayed

considerably higher serum glutamic-oxalacetic acid transaminase (SGOT) activity than normal. The authors are of the opinion that this is probably due to damage to transaminase-rich organs such as the liver. Injection of B_{12} had no effect on the SGOT level, whereas supplementation with pyridoxine increased the serum level of the enzyme as expected. A larger supply of vitamin B_6 did not increase the SGOT and, furthermore, brought about a significant decrease of this enzyme and a slight increase in xanthurenic acid when a tryptophane load test was applied.

The authors consider that these findings confirm their thesis that deficiency of one vitamin can create disturbances in mechanisms involving other vitamins. They point out further that disturbances of the endocrines may occur which have far reaching effects. The work of S. D. J. Yeh and Chow (*Am. J. Clin. Nutrition* **7,** *426 (1959)*) is cited which demonstrated that vitamin B_6 deficiency brought about a decrease in the secretion of gastric juice and intrinsic factor as well as an increase in gastric acidity. Correlated with these changes were dysfunctions of the adrenals.

INJURY, GROWTH, AND NITROGEN BALANCE

A decrease in growth rate as well as the nitrogen deficits which occur following an experimental injury can be prevented by adequate nutrition during the post-injury period.

In spite of many years of intensive work on the relationship of trauma to nitrogen balance and dietary intake (*Nutrition Reviews* **16,** *196 (1958)*), there are still some basic problems in this area which have not been solved. In the last few years a completely water-soluble diet of well defined chemical constituents has been developed. S. M. Birnbaum, J. P. Greenstein, and M. Winitz (*Arch. Biochem.* **72,** *417 (1957)*) have shown that such a diet will allow animals to grow and attain positive nitrogen balance.

Using such a diet, H. M. Radwin, M. Rechcigl, Jr., and Greenstein (*Surgery* **48,** *687 (1960)*) designed a group of studies in which they measured the growth and nitrogen balance of young growing rats maintained on this well defined diet before and after an injury. These investigators administered the standard injury by crushing the upper portion of the right hind limb of an anesthetized animal for 15 seconds with a rubber shod intestinal clamp. This, they claimed, invariably produced a fracture of the femur.

These investigators carried out four different groups of studies in which the animals were maintained on the water-soluble regimen. In all studies except ad libitum feeding the animals were tube fed four times a day at 9 A.M., 1 P.M., 5 P.M. and 9 P.M. Thus the animals received their total daily allotment of nutrients in four divided doses. One group of animals received 18 ml. of the diet per day both before and after the trauma. A second group received 18 ml. of the diet per day before the trauma and 24 ml. of diet per day following the trauma. The third group, not subjected to injury, received 18 ml. of diet per day during the entire study. The animals receiving either 18 or 24 ml. of diet prior to injury had an average weight gain of 10.2 ± 0.9 g. for the four-day study period. Following the injury, however, the animals on the lower oral intake had gained only 12.3 ± 0.2 g. for the ten-day period, while those receiving the 24 ml. of solution had gained 27.4 ± 1.3 g. In comparison, the animals which were not injured and were fed 18 ml. of diet throughout the study had gained 11.2 g. $\pm$ 1.4 for the four-day period before injury; during the ten days after injury they had gained 18.6 ± 0.6 g. Thus, the

injured animals gained less in the postoperative period than the non-injured animals receiving the same low dietary intake, but those which had increased dietary intakes (24 ml. per day) gained more in spite of being injured. In comparison, a fourth series of experiments were carried out in which the animals were allowed to eat the same fluid diet ad libitum both before and after the injury. It was found that before injury the animals ate on the average of 25 ml. of diet per day; after injury there was no appreciable difference in the intake. Before trauma the injured animals gained 13.2 ± 2.2 g. per four day period while the animals which were not to be subjected to injury gained 15.6 ± 0.8 g. However, the injured animals had gained 34.2 ± 4.4 g. in ten days and the non-injured animals had gained 32.8 ± 6.6 g. during a similar period. Thus it appears that there is no marked depression in voluntary food consumption as a result of the injury. Moreover, the injury did not cause a marked decrease in growth provided the animals were receiving 24 ml. of diet per day (an amount similar to that which they would consume on an ad libitum regimen). The difference in nitrogen balance (preinjury − postinjury) of the group of animals receiving 18 ml. of diet both before and after surgery was −31.6 ± 4.5 mg. per day. However, the group receiving 18 ml. of diet before injury and 24 ml. after injury had reduced the nitrogen deficit to only 9.8 ± 5.8 mg. per day.

From these studies it appears that the nitrogen deficit which occurs following injury can be minimized by giving an adequate diet during this period; also the growth rate of the injured animals need not differ greatly from the non-injured animals maintained upon a good intake.

These investigators also noted that there appears to be a significant difference in the way the animals utilize their nutrients, depending on the manner in which they are supplied (*e.g.* ad libitum feeding compared to tube feeding four times daily). The weight gain was 24.8 per cent greater and the nitrogen retention 87.3 per cent higher in the ad libitum-fed group than in the two series of force-fed animals. Such effects have been noted earlier (*Nutrition Reviews* **18,** *334 (1960)*) in comparing the changes in body composition of rats allowed to eat ad libitum and those getting similar amounts of food in large units.

FLUORIDE CONTENT OF SKELETAL TISSUES

New data have been reported on the relation of fluoride intake to hardness, radiographic analysis, and fluoride distribution in teeth, and on the effect of fluoride on bone composition. The data, although not extensive, do not support the concept that high fluoride intake promotes harder teeth.

Previous reviews have considered the relationship of fluorides to oral health and to the deposition of fluorides in the skeleton (*Nutrition Reviews* **15,** *145 (1957)*; **17,** *133 (1960)*). Increasing amounts of information are becoming available on the distribution of fluoride in various areas of the skeleton and on the gradients within individual tissues. S. H. Yoon, F. Brudevold, D. E. Gardner, and F. A. Smith (*J. Dent. Res.* **39,** *845 (1960)*) have studied the distribution of fluorides in various layers of teeth of different ages from several geographical areas where water supplies contained from 0.1 to 5.2 p.p.m. fluoride.

The concentration of fluoride in the outer surface layers of enamel was always much greater than for the deeper layers. A decrease in fluoride concentration occurred very rapidly as the distance from the surface increased, with the main bulk of the enamel having a consistently low value, about 0.1

of the surface layer. This gradient occurred at all levels of fluoride ingestion, although the fluoride concentration in any given part of the enamel increased with higher levels of fluoride ingestion. The concentration of fluorides in surface enamel increased with age at all levels of fluoride ingestion; these post-eruptive increases were most readily demonstrable in communities where the level of fluoride in the water was low and least conspicuous where the level of fluoride in the water was higher. No relationship of age to fluoride concentration was observed in the subsurface enamel. Higher fluoride concentrations were always observed in the layers of dentin adjacent to the pulp and in the surface layers on the root surface than in the inner dentin. Increasing fluoride concentrations with age were noted in the pulpal layers of dentin and in the root surface. Greater increases expressed in per cent were noted in the dentin than in the enamel as a result of the ingestion of higher levels of fluoride in the water. While the average fluoride concentration in the dentin was routinely higher than the average concentration in the enamel, the concentration of fluoride in the surface layers of enamel was always higher than the average dentin value.

Yoon *et al.* (*J. Am. Dent. Assn.* **61,** *565 (1960)*) have also studied the concentrations of fluoride in various layers of human teeth in relation to the concentrations in various layers of the adjacent bone. These values were obtained from specimens from 11 cadavers and from one fresh dog mandible. In descending order of magnitude the fluoride concentration expressed in parts per million of the ash weight were the following samples: surface layer of pulpal dentin (2,711); surface layer of cementum (2,390); external layer of compact bone (1,490); cancellous bone (1,205); lamina dura (1,165); apical portion of the root dentin (1,084); surface layer of enamel (584); and main portion of the enamel (62).

Microradiographic studies were conducted with sections of 13 permanent fluorosed teeth collected from residents in Clovis, New Mexico, Colorado Springs, Colorado, Doland, South Dakota, and Post, Texas where the water supplies varied in fluoride content from 2.2 to 5.0 p.p.m. (E. Newbrun and Brudevold, *Arch. Oral Biol.* **2,** *15 (1960)*). Most of these teeth had areas of radiolucency in the enamel varying from small restricted regions to extensive involvement. In all cases the outer surface was well-calcified and highly radio-opaque as was the deep layer of enamel adjacent to the dentin. With the lower concentrations of water fluoride, the radiolucent, hypocalcified areas were in the third of the enamel just below the surface layer. At the highest level of fluoride, the outer ½ to ¾ of the enamel below the surface was affected. These radiolucent characteristics appeared to be most extensive in the striae of Retzins in the interprismatic material. Unfortunately comparable teeth from communities with insufficient fluoride or with optimal levels of fluoride in the 0.7 to 1.2 p.p.m. range had not been sectioned and radiographed. It would be interesting to know what the radiographic transmission characteristics were like for fluoride-deficient teeth as well as for teeth with optimal fluoride concentrations.

Thirty permanent teeth were collected for microhardness studies from individuals who had grown up in Alamosa, Colorado, Colorado Springs, Colorado, Doland, South Dakota, and Post, Texas, where the water supplies varied in fluoride concentration from 1.5 to 5.0 p.p.m. (Newbrun, *Arch. Oral Biol.* **2,** *21 (1960)*). The average microhardness values from these four communities were 305, 355, 268, and 396 units, respectively. Comparable values for Birmingham, Alabama, Binghamton, New York, and Schenectady, New York were 367, 369, and 374, respectively, where the water contained between 0.00 and 0.15 p.p.m. fluoride. The value of 268 for Colorado Springs was for three teeth from one individual which were

conspicuously soft. This value probably could be eliminated from the series as irrelevant by reason of the nature of the sample. The hardness value for the teeth from Post, Texas where the water supply contains 5.0 p.p.m. fluoride was the only one that was significantly higher than those from the low-fluoride communities. A strong inverse correlation was observed between the degree of mottling and the microhardness value. As in the previous paper, it is unfortunate that no teeth were included in the series from communities where the level of fluoride ingestion was optimal. Although this series is small and has this serious omission, these data raise a real question about the validity of the prevalent hypothesis that the higher caries resistance of teeth containing optimal amounts of fluorides may be related to their greater hardness.

A comprehensive study has been conducted to determine the relationship of a wide range of skeletal fluoride concentrations to the amounts of other components (I. Zipkin, F. J. McClure, and W. A. Lee, *Arch. Oral Biol.* **2,** *190 (1960)*). Sixty-nine samples of human bone, comprising iliac crest, rib and vertebra, were obtained from 23 individuals, 26 to 90 years of age, who had consumed drinking water containing up to 4.0 p.p.m. fluoride for ten to 87 years prior to their death. The quantity of fluoride in the bone ash varied from about 700 to 8,000 p.p.m., with the values in direct proportion to the amount of fluoride in the drinking water consumed. Calcium and phosphorus concentrations and calcium-phosphorus ratio were unaffected over the tenfold range of fluoride concentration. There was a slight tendency for bone ash to increase moderately with increased fluoride concentration. As bone fluoride increased, there was an increase in magnesium concentration, a slight but consistent decrease in sodium concentration and a minor generalized decrease in potassium concentration. With increasing bone fluoride, there was a substantial decrease in carbon dioxide content in all bones and a striking decrease in citrate content. The authors postulate that the decreases in sodium, potassium, carbon dioxide, and citrate in the bone ash may be attributable to the fact that these components are presumed to be largely surface oriented on the hydroxyapatite crystals of the bone mineral and may be exchanged for fluoride at higher levels of fluoride ingestion. Most of the individuals in this survey were advanced in age at which time exchange reactions at bone surfaces predominate over remodelling. This state would favor surface deposition of fluoride ions rather than incorporation into the crystals.

These studies provide additional information about the metabolism of fluoride and its influence on the skeletal structures. Obviously the studies on radiolucency and microhardness are preliminary in nature and need to be extended greatly in scope and variety of teeth sampled. Comparable studies need to be done on the physical characteristics of bone parallel to the intensive and excellent surveys of the relationship of bone fluoride to other mineral components.

NOTES

Liver Enzymes in Glycogen Storage Disease

Von Gierke's disease is a result of an inborn error of metabolism which allows a massive storage of glycogen in the liver and perhaps other tissues. Fifteen per cent or more of the weight of the liver in such individuals has been reported to be glycogen. In earlier reviews an absence of glucose-6-phosphatase in the livers of such patients has been reported (*Nutrition Reviews* **14,** *63 (1957)*).

Recently, G. Weber and E. R. Harpur (*Metabolism* **9,** *880 (1960)*) have reported measurements of the activity of various enzymes involved in the metabolism of carbohydrates in a patient with glycogen storage disease. The source of the enzymes was a liver biopsy taken from an 11 month old male infant who showed various signs and symptoms associated with glycogen storage disease. The child had a fasting hypoglycemia and a marked lipemia; the fasting liver glycogen was 8.06 g. per cent. The administration of epinephrine caused no increase in the blood sugar. The liver biopsy did not possess either glucose-6-phosphatase or glucose-6-phosphate dehydrogenase activity.

However, the activity of phosphoglucomutase activity was increased (142 per cent), along with activity of the following enzymes in the liver: phosphohexoseisomerase (468 per cent); fructose-1,6-diphosphatase (213 per cent); and lactic dehydrogenase (325 per cent). While the absence of glucose-6-phosphatase activity could be an important factor in explaining the increased accumulation of glycogen in the liver, it need not be the whole answer. The absence of glucose-6-phosphatase activity along with a lack of glucose-6-phosphate dehydrogenase activity would allow the glucose to go mainly into glycogen. The increased activity of the phosphoglucomutase, along with the decrease of the other two enzymes discussed, would tend to drive glucose-6-phosphate toward glycogen.

These investigators believe that there is an elevated gluconeogenesis suggested by the great increase in the activity of the fructose-1,6-diphosphatase and the phosphohexoisomerase activities. They also believe that all these factors may tend to increase the size of the glucose-6-phosphate pool which would increase the amount of glycogen in the liver.

While these enzyme changes may explain why there is increased liver glycogen in patients with Von Gierke's disease, it does not explain the different type of glycogen which has been reported in certain of the cases (*Nutrition Reviews* **11,** *154 (1953)*). This latter effect may be the result of still further enzymatic abnormalities.

Fatty Acid Chain Length and Essential Fatty Acid Deficiency

Long-chain triglycerides enhance essential fatty acid deficiency whereas medium-chain triglycerides have little effect on the disease.

With continuing research on essential fatty acid deficiency it has become increasingly apparent that the requirement for essential fatty acids is dependent on a great many factors, including the composition of the dietary fats other than those containing the essential fatty acids. Thus, J. J. Peifer and R. T. Holman (*J. Nutrition,* **68,** *155 (1959)*) found that the requirement of rats for essential fatty acids is partially dependent on the saturated fatty acid content of the diet.

In a similar study, H. Kaunitz, C. A. Slanetz, R. E. Johnson and V. K. Babayan (*J. Am. Oil. Chem. Soc.* **36,** *322 (1959)*) investigated the nutritional properties of medium-chain saturated triglycerides (MCT), containing mainly fatty acids,

with chain lengths from six to 12 carbons and long-chain saturated triglycerides (LCT) containing mainly fatty acids with 14 to 18 carbons. Weanling rats were fed either a fat free diet or the same diet with 20 per cent of the dextrose replaced by the MCT or LCT. Each group received supplements of 0.0, 0.1 or 2 per cent of a linoleic acid concentrate from safflower oil. It was found that with the 2 per cent linoleate supplement somewhat better growth was obtained with diets containing 20 per cent fat of either type. However, in the absence of linoleate, rats on the MCT-containing diets had better growth rates than those on the LCT-containing diets. The authors concluded that the MCT decreased the requirement for linoleate while the LCT increased it.

In a continuation of these studies, the same authors (*J. Nutrition* **71,** *400 (1960)*) checked organ weights of animals on the same diets. The linoleic acid-deficient, animals in all groups had enlarged livers, adrenals, kidneys, heart ventricles, and testicular fat bodies. Thymus weights were subnormal. Although the type of fat in the diet generally had no effect on the organ weights of linoleic acid-sufficient rats in the deficient groups, those on the LCT-containing diets had higher adrenal weights and enlarged fat bodies as compared with those on the fat-deficient and MCT-containing diets.

As in the previous study, the conclusion was that the medium-chain length triglycerides had no influence on the course of essential fatty acid deficiency, but that the long-chain triglycerides enhanced it. These results generally agree with those of Holman and others, but the mechanisms behind them must await further experimentation.

Recent Books

Strontium 90 in Human Diet in the United Kingdom 1959. Agricultural Research Council Radiobiological Laboratory Report No 3. Her Majesty's Stationery Office, London. Agents in U.S.: British Information Services, 45 Rockefeller Plaza, New York 20, N. Y. Price 70¢.

Nutrient Requirements of Poultry. No. 1, Revised 1960. Nutrient Requirements of Domestic Animals—A Report of the Committee on Animal Nutrition. Publication #827. National Academy of Sciences—National Research Council, Washington, D. C. Price $1.00.

Nutrition et Alimentation Tropicales. Vols I, II, III. Marseille, France, 4 Octobre-15 Decembre 1955. Organization Des Nations Unies Pour L'Alimentation et L'Agriculture. Rome 1957. Pp. 1–2040.

The Composition of Foods. Special Report Series No. 297. By R. A. McCance and E. M. Widdowson. Published by Her Majesty's Stationery Office, Atlantic House, Holborn Viaduct, London, E.C. 1. Pp. 252. Price £ 1 10 s. Od. net.

BOARD OF TRUSTEES

W. Gardner Barker, *President, Thomas J. Lipton, Inc.*

*Claude T. Bissell, *President, University of Toronto*

*F. G. Boudreau, *President, Milbank Memorial Fund.*

William T. Brady, *Chairman, Corn Products Company.*

Charles S. Bridges, *President, Libby, McNeill & Libby*

G. Buitoni, *President, Buitoni Foods Corp.*

*Cason J. Callaway, *Hamilton, Georgia*

*O. C. Carmichael, *Biltmore, North Carolina*

Thomas P. Carney, *Vice President, Research, Development & Control, Eli Lilly and Company*

Harold W. Comfort, *President, The Borden Company*

Herbert C. Cornuelle, *Pres. Dole Corp.*

W. T. Crighton, *General Manager, Producers Creamery Company*

Wesley M. Dixon, *Chairman, Container Corporation of America*

*Lee A. DuBridge, *President, California Institute of Technology*

Joseph Eichberg, *President, American Lecithin Co., Inc.*

*Conrad A. Elvehjem, *President, University of Wisconsin*

Thomas C. Fogarty, *President, Continental Can Co., Inc.*

Edgar J. Forio, *Senior Vice President, The Coca-Cola Company*

*Clarence Francis, *New York, New York*

T. R. Gamble, *President, Pet Milk Company*

Daniel F. Gerber, *President, Gerber Products Co.; Vice President, The Nutrition Foundation, Inc.*

George A. Graham, *Secretary Treasurer, Charles B. Knox Gelatine Co., Inc.*

John A. Grammer, *Executive Vice President, Beech-Nut Life Savers, Inc.*

Henry J. Heinz, II, *Chairman, H. J. Heinz Co.*

*Rev. Theodore M. Hesburgh, C.S.C., *President, University of Notre Dame*

Frank C. Hildebrand, *Vice President, General Mills, Inc.*

*Herman E. Hilleboe, *Commissioner of Health, State of New York*

*Frederick L. Hovde, *President, Purdue University*

Porter M. Jarvis, *President, Swift & Company*

*James R. Killian, Jr., *Chairman, Massachusetts Institute of Technology; Chairman of Board of Trustees, The Nutrition Foundation, Inc.*

*Charles Glen King, *Executive Director, The Nutrition Foundation, Inc.*

*Grayson L. Kirk, *President, Columbia University*

R. Newton Laughlin, *President, Continental Baking Co.*

J. Preston Levis, *Chairman, Owens-Illinois*

R. G. Lucks, *President, California Packing Corp.*

W. F. McLean, *President, Canada Packers, Limited*

William H. McLean, *President of Chemical Division, Merck & Co.*

Oscar Mayer, *Chairman of the Board, Oscar Mayer & Co.*

Charles G. Mortimer, *Chairman, General Foods Corporation: President, The Nutrition Foundation, Inc.*

Milton C. Mumford, *President, Lever Brothers Co.*

W. B. Murphy, *President, Campbell Soup Co.*

H. Earle Muzzy, *Vice Chairman, The Quaker Oats Co.*

William F. Oliver, *President, The American Sugar Refining Company*

W. W. Paddon, *President, Sunshine Biscuits, Inc.*

*Thomas Parran, *President, Avalon Foundation*

*Maurice Pate, *Executive Director, United Nations Childrens Fund*

Philip W. Pillsbury, *Chairman, The Pillsbury Company*

J. G. Pleasants, *Vice-President in charge of Research and Development, The Procter & Gamble Company*

B. E. Richmond, *President, Richmond-Chase Company*

Lloyd E. Rigler, *President, Adolph's, Ltd.*

Lyle C. Roll, *President, Kellogg Company*

R. T. Ryan, *Executive Vice President, Welch Grape Juice Co., Inc.*

Elbert M. Shelton, *Secretary, The Baker Laboratories Inc.*

Vernon Stouffer, *President, The Stouffer Corporation*

Roy J. Sund, *President, American Can Company*

Henry Weigl, *President, Standard Brands, Incorporated*

J. Huber Wetenhall, *President, National Dairy Products Corporation*

F. R. Wilcox, *General Manager, Sunkist Growers*

E. W. Wilson, *Executive Vice President, Armour & Company*

R. D. L. Wirth, *President, Red Star Yeast and Products Co.*

H. J. Wolflisberg, *President, The Nestle Company, Inc.*

Horace L. Sipple, *Secretary* John Holmes, *Honorary Trustee*

---

* Representative of the public.

THE NUTRITION FOUNDATION

The Nutrition Foundation was organized by food and related manufacturers in December, 1941, as a sincere expression of their interest in scientific progress and human health. The basic purposes of the Foundation are: (1) the development of a comprehensive program of fundamental research, providing basic information in the science of nutrition; and (2) the support of educational measures that will assist in making the science of nutrition effective in the lives of present and future generations.

The publication of NUTRITION REVIEWS has been undertaken to enable professionally trained people to keep abreast of current progress and to have available an unbiased, authoritative review of the current research literature in the science of nutrition.

The Editorial Staff has been carefully selected so that the publication will merit, in reasonable degree, the confidence of the medical profession and others who work directly with the public in the field of nutrition. The service provided by the publication is distinct from that of an abstract journal, a review for laymen, or an extensive scientific review. Since the reviews as originally prepared by a member of the Editorial Staff are frequently modified by special referees and may be further modified by the Editorial Office in preparing final copy for the press, the Editorial Committee feels that it is preferable not to affix the names of Editorial Staff members to individual reviews. There is thus a high degree of group responsibility on the part of the Editor and Associate Editors.

Signed articles do not necessarily represent the opinion of the Editorial Staff.